SOME STUDIES ON NEMATODE BEHAVIOUR

ABSTRACT

THESIS SUBMITTED TO
THE ALIGARH MUSLIM UNIVERSITY, ALIGARH
FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
ZOOLOGY

BY
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DEPARTMENT OF ZOOLOGY
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ABSTRACT

In the present work an attempt was made to elaborate and explore in detail, the reproductive behaviour of some soil-inhabiting nematodes. Experiments on sex attraction, copulatory behaviour, copulatory senses, ageing and reproduction, and orientation were conducted on the following three species of nematodes: i) Chiloplacus symmetricus, ii) Curznema lambdiensis and, iii) Rhabditis sp.

Observations on sex attraction in Chiloplacus symmetricus showed that only the males were attracted to female secretions, females and fourth-stage male juveniles did not respond. Males showed no significant attraction towards male or fourth-stage female juvenile secretions. The females did not respond to either male or fourth-stage female juvenile or their own secretions. Similarly, fourth-stage male juveniles were unresponsive to any secretions. Orientation of males to sex attractants involved both klinokinesis and klinotaxes, the final approach being a direct movement. Copulation took place when the male coiled its tail around the female and the spicules located the vulval opening. The spermatozoa after deposition in the uterus moved upwards and accumulated in the spermatheca. Copulation lasted from half a minute to nearly 45 minutes.

A study of the factors influencing sex attraction in Chiloplacus symmetricus revealed that movement of males to attractant sources was highly variable depending on the experimental techniques employed. In Petri dish experiments, fewer females produced a significant male response than in the mickey mouse traps and also the incubation period needed by the females to produce a response from the males was lesser in the former experiment.

Attraction was evident earlier in the Petri dish experiments than in mickey mouse traps. Within each type of experiment also, sex attraction varied with the number of females at the attractant source, period of incubation, time of observation, thickness of agar and the concentration of agar. Generally, five females incubated for 18 hr resulted in a good response of males in the Petri dish experiment but at least 50 females incubated for 18 hr were required for the mickey mouse trap. Attraction was evident towards five females in the former experiment in 2 hr and towards 50 females in the latter experiment also in 2 hr. 1,2 and 4 mm thick layers of agar did not produce any change in attraction but in 8 mm thick agar attraction decreased significantly. Agar concentration of 4 and 8% inhibited sex attraction in both sets of experiments while there was no significant difference in 1 and 2% agar. Light produced no significant difference in attraction in either experiment.

The sex attraction of ageing males and females of C. symmetricus showed that this phenomenon was dependant on the age and reproductive state of the worms. All age-groups of virgin males were responsive to all age-groups of virgin females except 22 day old males to 18 and 22 day old females. The response of young males to older females decreased gradually and similarly the response of ageing males to younger females also decreased gradually. Attraction between virgin males and non-virgin females showed that males of all age-groups were attracted to 10 day old non-virgin females and all except 22 day old males were also attracted to 14 day old females but males did not respond to older females. Males of all non-virgin age-groups showed a positive response to virgin females of all age groups except 22 day old males to 22 day old females. In non-virgin males to non-virgin females, 10 day old females were attractive to males of all age groups and only 10 and 14 day old males were attracted to 14 day old females. Females of other age-groups were not attractive.

In Curznema lambdiensis males did not attract males and similarly females did not attract females. Young virgin males responded to young virgin females but not to old virgin females. Young virgin females, however, responded to both young and old virgin males. Virgin males were not attracted to non-virgin females

but non-virgin males were attracted to virgin females. Non-virgin females showed a positive response to virgin males and virgin females also responded to non-virgin males. Attraction of males to females and females to males increased when the number of attractant worms increased to 50 from ten but a further increase did not produce a corresponding increase in attraction. When both males and females were put at the attractant source, the attraction of females increased from female: male ratio 1:50 to 20:50 but declined thereafter to increasing ratios. Males, however, did not show any similar increase to male: female ratios and attraction gradually decreased from 1:50 to 50:50 male: female ratio.

From the studies on the copulatory behaviour of Curznema lambdiensis it was concluded that copulation involved three distinct steps: i) attachment to female and location of the vulva, ii) penetration by the spicules and, iii) insemination. The bursa aided in gripping the female while the spicules located the vulval opening but did not take part in channelising the sperm from male to female reproductive tracts. The build up of internal pressure to release sperm was accomplished by shortening and swinging of the body in wide arcs. Females continued feeding during copulation. The mean number of copulations per day varied from 3 to 7.2 and the sperm transferred per day from 61 to 176. On an average 20-33 sperm were transferred per copulation per day. In its life span,

a male copulated 15 to 32 times and transferred a total of 517-754 sperm. When males were isolated for more than two days, both the number of copulations and the number of sperm transferred decreased. The mean number of sperm transferred on the first copulation was maximum in two day old males while three and four day old males showed a significant decline. As the isolation period of the males increased, the time required for the first copulation also increased.

In ageing virgin females of Curznema labdiensis the number of oocytes released by the ovary was less than in copulating females. Unfertilized oocytes failed to develop an egg shell and usually ruptured in the uterus. The egg mass sometimes passed out of the body during vulval twitchings or was reabsorbed by the uterine walls. In old virgin females, the ovary gradually became vacuolated and then shrivelled up. In copulating females as many as 171 eggs were produced on the first day. Fertilization took place in the spermatheca but the oocytes first contacted the sperm in the oviduct. Eggs were laid in batches but in older females they were retained in the body and ultimately led to 'endotokia matricida'. Spermatozoa in virgin males began maturing by the end of the final moult and within a day filled the entire seminal vesicle. On the third day they began to degenerate. Such degenerate spermatozoa had either a condensed cytoplasm or their

outer layer became mammilated. The testis degenerated in the same way as the ovary. Normally copulating males did not accumulate sperm in their seminal vesicle. Degenerative changes started on the third day. The mean life span of virgin worms was 10 days while of non-virgins it was only 6.5 days. When ageing males were mated with young females or vice-versa, egg production gradually decreased. Similar results were obtained for egg production after the first copulation.

An analysis of the copulatory senses of C. lambdiebsis revealed that males, either virgin or non-virgin, could distinguish between an inanimate and an animate object but could not differentiate between dead and live females either on sterile agar or on agar with an attractant gradient. Freshly moulted males copulated regularly over the entire three day period but maximum sperm were transferred during the first copulation. Two day old males copulated rapidly and a greater number of sperm were transferred per copulation on the first day than on the second and third day. On the second and third day the intervals between copulations increased In alternately isolated and copulating males, copulations occurred at a faster rate than normally and the number of sperm transferred was also greater.

Movement of males of Rhabditis sp was random on plain sterile agar as was indicated by the high correlation coefficients

but in the presence of female secretions, males showed a bias pattern of movement (no correlation) and tended to accumulate at the source of attractant. Males orienting to a point source showed more turnings and asymmetric movements than when orienting to a source 2 mm in diam. In the former case, tracks were often extremely circuitous. The movement of males from the centre of an attractant area was highly variable and in most cases, the males orientated initially towards a point between two attractant sources and only when near the source did they move directly. However, those males that moved into the area between two attracting sources at the periphery of the circle, showed very tortuous tracks and were captured by an attractant source only when they came very close to it. Males responding to attractants showed preferential movement and most aggregated at the five female source on both the attempts. The maximum number of males showing the same response on both the trials was at the five female source. However, not more than 50% of the males showed the same response on both the trials. Analysis of the locomotory characteristics in attractant and non-attractant zones of agar revealed that sex attractant inactivated males i.e., decreased their wave frequency. The wave length increased but the amplitude remained constant.

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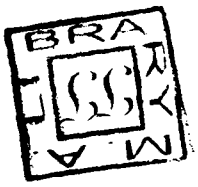

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5 GENETICS

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SUPERVISOR:

This is to certify that the entire research work which is being presented in the thesis entitled "Some studies on nematode behaviour" by Mr. Irfan Ahmad is original and was carried out under my supervision. I have allowed Mr. Ahmad to submit it to the Aligarh Muslim University in fulfilment of the requirements for the degree of Doctor of Philosophy in Zoology.

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CONTENTS

INTRODUCTION

In the last few decades, extensive research work has been done on the taxonomy, morphology, biology, physiology, ecology etc. of nematodes. The diversity within this group and the wide variety of ecological niches which they occupy has led to rapid advances in the study of fundamental and economically important aspects of Nematology. The study of nematode behaviour which remained largely neglected has attracted attention only in the recent past. Despite this, the primary phase of descriptions of behavioural activities of nematodes has almost passed and the behaviour is now being studied in relation to ecology, physiology and functional morphology. Very recently, scientists have started looking for clues to interpret behaviour at the molecular level.

Earlier studies on animal behaviour have largely been carried out on protozoans and insects. Jennings (1904) has emphasized the role of the physiological state of an individual in relation to its behaviour and showed that both are interdependant on one another. The behaviour of an animal in general has been defined as a response to a stimulus (Carthy, 1958; Diether & Stellar, 1958; Cloudsley-Thompson, 1961) and this in essence forms the total behavioural component in lower animals. Insects with their complex reactions and social behaviour enjoy hierarchical dominance in terms of behavioural patterns. Still higher come

the vertebrates displaying instinctive behaviour and finally man "who alone among the creatures can look upon himself and become the object of his own thoughts, can distinguish the world he knows from himself as knowing it" (Knox). It, therefore, becomes apparent that the degree of specialization and diversity of behaviour is limited to the potentialities of the nervous system of an individual. Herrick (1962) has divided behaviour into three grades, viz., somatic behaviour, visceral behaviour and genetic behaviour and has stated that they differ only in their manifestations but conform to the basic physiological processes. Detailed studies on the orientations of animals have been described by Fraenkel & Gunn (1961) particularly on kinesis and taxes. They (l.c.) have illustrated and differentiated between the different responses and attempted to standardize the basic terminologies used in describing animal behaviour. Besides, Gunn & Kennedy (1945), Soulairac (1949) and Ewer & Bursell (1950) have contributed significantly towards the classification of animal behaviour.

Nematode behaviour though studied earlier was centred on areas of biomedical and agricultural importance. The study of nematode behaviour in the broader perspective of analysing responses in relation to other animals and the functional morphology of the nematode itself is a relatively young subject. Even then, numerous responses analogous with other invertebrates have already been described by various workers. Further, ultrastructural

studies have brought to light the complex nature of the anterior sense organs of nematodes and provided clues for their possible mechanism of functioning (Wright, 1976; McLaren, 1976). Wallace (1961) provided evidence for orthokinesis in Ditylenchus dipsaci, which led to its accumulation in fine sand particles while Blake (1962) suggested that this species located host roots by klinokinesis and Klingler (1963) studying the response to CO_2 gradients concluded that orientation occurred by klinotaxes. The diversities of nematode behaviour are apparent, however, its interpretations from the viewpoint of functional organization are still in the process of study. The nature of the sense organs and their practical functional implications was highlighted by Croll (1967a) when he stated that during movement, the amphids, which are supposed to be chemoreceptors, did not lie side by side but at right angles to the plain of movement. Hence, although bilaterally paired sense organs may be present, orientation by tropotaxes may not always be possible. But when swimming in water, Chromadorina viridis provided evidence for tropotactic orientation under varying intensities of light (Croll, 1967a). Nematode response may be a kinesis, i.e., a non-directional movement in response to a variation in the stimulus without orientating the body axis to the source of stimulation or a taxes, i.e., a directional response where the body axis lies in line with the stimulating source. Kinesis is further divided into an orthokinesis which is an undirected response

and in which activity depends on the intensity of stimulation and klinokinesis in which the rate of turning depends on the intensity of stimulation. Similarly, taxes is divided into klinotaxes, i.e., where orientation is by comparison of stimulus intensity by regular lateral deviation at successive time intervals, or tropotaxes, i.e., where orientation is by simultaneous comparison of intensity of stimulation on each side. Work with Caenorhabditis elegans mutants has revealed that lack of response to chemical gradients was due to morphological abnormalities in a sensory neuron of the amphids (Ward, 1976). While some mutants did not respond to chemicals, others avoided substances that were normally attractive and most were defective in response to more than one stimulant thus exhibiting a pleiotrophic effect (Riddle, 1978). By identifying genes responsible for the development of a particular system or structure, interpretation of behaviour on the basis of genetics became possible.

The complexity displayed in nematode behaviour seems to be correlated with the degree of development of the sense organs and the ability of perception since behaviour is assessed in terms of activity and locomotory patterns (Green, 1966; Croll, 1967a; 1970; 1971; Azmi & Jairajpuri, 1978) and activity is a consequence of sensory input (Croll, 1972). Numerous sense organs have been described giving evidence for a variety of sensory functions. From the study of setae of marine nematodes Croll & Smith (1974)

concluded that these acted as mechanoreceptors. Cephalic papillae in the filarial nematode Dipetalonema viteae were also thought to be mechanoreceptors (McLaren, 1972). Baldwin & Hirschmann (1973) related cephalic papillae to mechanoreception and labial papillae to chemoreception in Meloidogyne incognita principally because in the former nerve endings lay embedded just below the cuticle while in the latter they had access to the exterior through a small pore. Robertson (1975) suggested a possible gustatory role played by a sensory area in the odontophore of Longidorus leptocephalus and Xiphinema diversicaudatum but commented that their structures were not typical of chemoreceptors found in other nematodes and invertebrates. Burr & Webster (1971) while studying the ultrastructure of the pigment spots of Oncholaimus vesicarius concluded that they were not photoreceptors but acted as shades for photoreceptive microvilli located in the amphids. The fine structure of the photoreceptors of Deontostoma californicum revealed that pigment granules might act as shields around the photosensory body (Siddiqui & Viglierchio, 1970). The role of the posterior sense organs, viz., phasmids in the behavioural coordination of nematodes has not been elucidiated. In fact, detail structures are known only in a few species of animal parasites (Muller et al., 1970; McLaren, 1972). The possibility of phasmids functioning in association with the anteriorly located amphids was unlikely as C. elegans mutants with

a blistered tail orientated normally to a chemical gradient (Ward, 1973).

Besides possessing elaborate structures for chemo-, mechano- and photoreception, nematodes can perceive and respond to temperature gradients also. El-Sherif & Mai (1969) believed that Pratylenchus penetrans and D. dipsaci could detect temperature gradients as small as 0.033°C across 4 cm, while Croll & Smith (1972) showed that Ancylostoma tubaeforme larvae responded positively to a heat gradient and emphasized its significance with respect to the mechanism of infection of the parasite. Wallace (1961) suggested that D. dipsaci had a temperature preference of about 10°C as they tended to accumulate at this temperature. However, Croll (1967b) suggested that temperature preference was determined by the previous storage temperature of the nematode.

A large number of factors both internal and external influence behavioural characteristics of nematodes. Ageing is one important factor determining the outcome of a response. The activity of C. briggsae declined with age (Zuckerman et al., 1971) and in C. elegans the rate of oviposition and vulval contractions decreased (Croll, 1975). In Turbatrix aceti there was a decrease in fecundity with age (Kisiel & Zuckerman, 1974). Age related changes in C. briggsae produced an increase in osmotic fragility and

specific gravity (Zuckerman *et al.*, 1971; 1972) but these changes did not occur in virgin *T. aceti* (Kisiel *et al.*, 1975). In *Panagrellus redivivus* increase in osmotic fragility with age was specific for each sex, males succumbing earlier than females (Abdurahman & Samoiloff, 1975). Other changes which occurred in *C. elegans* included a linear decline in the rate of backwardly directed waves and doubling of the mean defaecation period (Croll *et al.*, 1977). Duggal (1978b) observed a decline in the rate of copulation and sperm transfer in *P. redivivus*. In *Strongyloides ratti* there was a decrease in both activity as well as infectivity with ageing larvae (Barrett, 1969), while in the cat hookworm *A. tubaeforme*, lipid levels and activity decreased with age under varying conditions of temperature, pH and osmotic stress (Croll, 1972) and Croll (l.c) concluded that activity was dependant on the lipid reserves of the larvae.

Pheromone behaviour has been described extensively in insects (Shorey, 1976) but in nematodes, so far, only the occurrence of sex pheromones is known (Greet *et al.*, 1968; Bone *et al.*, 1977). Nematode sex pheromones or sex attractants as they are more commonly known influence activity from only a very short distance while the alarm pheromone of the termite, *Nasutitermes exitiosus*, was found to be effective up to a radius of nearly 30 cm (Eisner *et al.*, 1976). However, Shorey (1976) stated that the distance of pheromone communication between animals was

variable and depended on a number of interacting factors. Not much work has been done on the physio-chemical and physical properties of nematode pheromones primarily because of the difficulty in obtaining a pure extract. Preliminary work has shown that the sex pheromones of Globodera and Heterodera spp., were volatile (Greet et al., 1968) and very labile and became ineffective after 24 hr (Green, 1966) in contrast to the alarm pheromone of the termite which remained attractive after 48 hr (Eisner et al., 1976).

In India, research in agricultural nematology started rather late, but the economic importance of the pests quickly led to the development of numerous nematological research centres all over the country. The study on plant nematodes in this department was initiated as early as 1955 under the able guidance of the late Prof. M.A. Basir. Subsequently, the Department of Zoology became a strong centre of nematological research but still pertaining only to the fundamental aspects of morphology and taxonomy. Behavioural aspects of nematodes were virtually untouched till Jairajpuri and Azmi started work in 1975. Their work, which included the study of activity, locomotion, reproductive and predatory behaviour, responses to salts and pH, temperature, light and ultrasonics was perhaps the first serious attempt, in this country, of analysing the nematode behavioural characteristics. Despite the general nature of the present problem, work

has been centred mainly on the study of the reproductive behaviour of some free-living species. Three different species were taken so that a comparison, if any, between species may be drawn, as well as the fact that a particular species may not be ideal to study all the aspects of behaviour. Hence, sex attraction, orientation and preliminary observations on copulation, factors influencing sex attraction and effect of ageing on sex attraction were studied in Chiloplacus symmetricus (Thorne, 1925) Thorne, 1937. In Curznema lambdiensis (Maupas, 1900) Thorne, 1961 which had a life span of about ten days (virgins) as compared to about 20 days of C. symmetricus, and in which the internal organization was more clear, detailed observations on the mechanism of copulation and sperm transfer were made as well as observations on the effects of age on the reproductive system and fecundity and analysis of the sex attraction behaviour was also carried out. Due to its short reproductive life and high rate of sperm transfer, C. lambdiensis also provided an opportunity to assess its copulatory senses. The isolated experiment on the orientation of male Rhabditis sp., was done with a view to analyse and elaborate the responses during orientation. Still, however, not all aspects of reproductive behaviour could be studied. Nevertheless, the utmost best has been tried to be achieved with the limited facilities at our disposal.

MATERIALS AND METHODS

Culturing nematodes

Chiloplacus symmetricus: Chiloplacus symmetricus was cultured in Malt-peptone agar in 5.5 cm diam Petri dishes. The medium consisted of 2.5 gms of Difco Malt, 2.5 gms of Bacteriological peptone (BDH) and 10 gms of agar dissolved in 1000 ml of distilled water. First the agar was dissolved in water by heating in a water bath. When dissolved but still molten, the dextrose and peptone was added to it and the medium was allowed to cool. The nematodes were washed in 1% mercuric chloride to eliminate surface bacteria and inoculated either singly or in groups into the agar. The unidentified bacterial growth on the surface of the agar was utilised as food by the nematodes. All specimens used in the present study were the progeny of a single gravid female.

Curznema lambdiensis and Rhabditis sp. : These rhabditid nematodes were cultured in plain agar supplemented with wheat flour. Before inoculation into the media, the nematodes were surface sterilised with 1% mercuric chloride solution, but as bacteria were also present in the gut of the nematodes, they also grew on the medium and provided additional diet for the nematodes.

Isolation of the nematodes from the culture medium: When required in large numbers, the nematodes were extracted from the

culture medium by the modified Baermann's funnel technique. The agar was chopped into small pieces, put in a small sieve lined with moist tissue paper and placed in a Baermann's funnel so that the water in the funnel touched the bottom of the sieve. The nematodes were collected from the bottom of the sieve after 12-24 hrs.

Preparation of plain agar: Plain sterile agar was prepared by dissolving the required amount of agar in distilled water heated in a water bath. When it was cool and in a molten state, one or two drops of lactic acid were added to prevent bacterial growth, and then poured into Petri dishes.

Experimental designs

Petri dish experiments: (Fig. 1) A plastic straw pipe 5 mm high, 5 mm in diam, with a small piece of filter paper glued to one end was filled with agar and the requisite number of nematodes were transferred into it. This was kept in the centre of a Petri dish containing 1% water agar, and a reference circle 2.5 cm in diam was drawn concentric with the straw pipe. After the required period of incubation, 5 worms which were to be tested for attraction were placed at various points on the reference circle and their distribution was recorded when desired. The distribution of the nematodes should be proportional to the area of each zone. Thus, in random distribution, more worms

should be present in the outer zone, lesser in the inner zone and practically none in the straw pipe or below it. Scores were obtained by summing up the products of the number of worms in each zone with their corresponding weighting factors. These scores were then converted into log scores (Table II). Areas and weighting factors of the three zones are given in Table I.

Mickey mouse chamber: (Fig. 2) This apparatus which was a modification of Samoiloff et al. (1973) technique consisted of three chambers, each 2.5 cm in diam, arranged linearly. The middle chamber was the inoculation chamber in which nematodes to be tested for response to attractants were placed. One outer chamber served as control which was left blank while the other outer was the test chamber in which nematodes producing attractants were placed. The test and control chambers were connected to the inoculation chamber by means of channels 2 mm wide and 10 mm long.

Observation chamber: This chamber was similar to that of Maertens (1975). A plastic ring 1 cm in diam was fixed on the coverslip of a metallic slide. An agar disc less than 1 cm in diam containing two or three pairs of young virgin adults was placed on a separate coverslip and this was inverted over the ring. The thickness of the agar was such that it did not touch the lower fixed coverslip. The edges were sealed with vaseline.

FIG. 1

The Petri dish apparatus for testing sex attraction.

FIG. 2

The modified mickey mouse chamber for testing sex attraction.

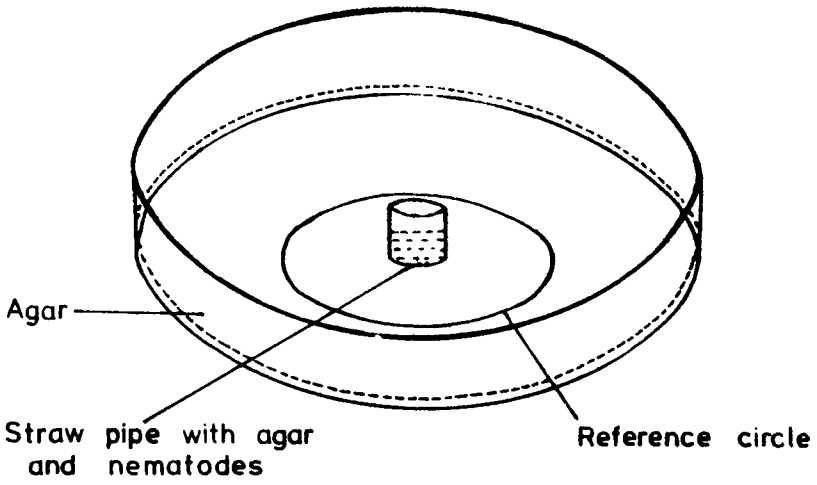

Agar
Straw pipe with agar and nematodes
Reference circle

FIG. 1

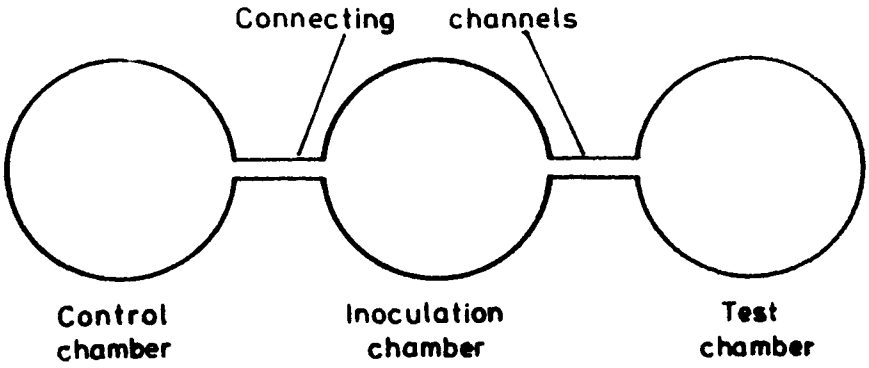

Connecting channels
Control chamber
Inoculation chamber
Test chamber

FIG. 2

TABLE I

Area and weighting factors for the zones marked on agar plates

	Block	Inner zone	Outer zone
Area (sq. mm)	20	471	1885
Weighting factor	94	4	1

TABLE II

21 positions males can occupy with their ranks and scores

Central block	Inner circle	Outer circle	Rank	Score	Log score
5	0	0	21	470	2.67
4	1	0	20	380	2.57
4	0	1	19	377	2.57
3	2	0	18	290	2.46
3	1	1	17	286	2.45
3	0	2	16	284	2.45
2	3	0	15	200	2.30
2	2	1	14	197	2.29
2	1	2	13	194	2.28
2	0	3	12	191	2.28
1	4	0	11	110	2.04
1	3	1	10	107	2.02
1	2	2	9	104	2.01
1	1	3	8	101	2.00
1	0	4	7	98	1.99
0	5	0	6	20	1.30
0	4	1	5	17	1.23
0	3	2	4	14	1.14
0	2	3	3	11	1.04
0	1	4	2	8	0.90
0	0	5	1	5	0.69

Study of tracks: Tracks inscribed on the surface of agar were studied in different concentrations of water agar. The agar was dissolved in water heated in water bath, poured into Petri dishes and was then allowed to cool. These dishes were kept in a thermostat for one to two hr to let the surface dry. Another method for studying tracks was that of Croll (1975) in which a little amount of molten agar was poured in a Petri dish, swirlled and the excess amount discarded. The thin layer of agar gave excellent results but could only be used for short periods.

Observations: All observations were made on stereoscopic binocular microscope but where a detailed study was required, a compound microscope was used.

Drawing and photographs: All drawings were made with the help of a camera lucida. Photographs were taken on a МБИ-6 microscope with a mounted Zorki 4 camera using a Fortepan panchromatic film.

All experiments were carried out at 28 $\pm$ 3°C.

LIST OF ABBREVIATIONS

T = Test chamber

C = Connecting channel

I = Inoculation chamber

O = Control chamber

um = μm

REPRODUCTIVE BEHAVIOUR IN NEMATODES

Nematodes living in the subterranean habitat and lacking the sensory organs of the higher invertebrates, have little chance of making contact with each other except at feeding sites. Without an elaborate mechanism of attraction, reproduction in obligate amphimictic species would almost entirely be dependant on chance encounters which would be too few at low population densities. Behaviour which promotes mate-finding seems essential as in most other obligate bisexual animals. The work on the reproductive behaviour has clearly shown that either both the sexes mutually attracted each other or only the females attracted the males. So far there has been no report of unilateral attraction by males only.

The first report of sex attraction in nematodes was that of Greet (1964) when he observed that both sexes of a free-living nematode, Panagrolaimus rigidus (Schneider, 1866) were attracted towards each other. Subsequently, Jones (1966) and Jairajpuri & Azmi (1977) noted male to female attraction in Pelodera teres (Schneider, 1866) and Acrobeloides sp., and Green et al. (1970) showed that Globodera rostochiensis (Woll., 1923) were not only strongly attracted to females but also showed a slight response towards males. Chin & Taylor (1969) studying sex attraction in Cylindrocorpus longistoma and C. curzii concluded that only males

of both the species were attracted to their respective females but interspecific attraction of males to females did not occur. However, there was positive attraction between different strains of the free-living nematode, Panagrellus silusiae (deMan, 1913) as reported by Cheng & Samoiloff (1971). The specific status of P. silusiae was doubted by Behme & Pasternak (1969) because mating between the 'C' strain of P. silusiae and the 'N' strain of P. redivivus (Linn., 1767) occurred with the production of fertile progency. Hechler (1971) based on the study of morphology, concluded that the synonomy of P. silusiae with P. redivivus as proposed by Ruhm (1956) was valid. Although interspecific attraction was not observed in C. longistoma and C. curzii but there is evidence (Green & Plumb, 1970) that the females of Heterodera/Globodera produced more than one kind of attractant and their males responded to more than one attractant. Males of Meloidogyne arenaria (Neal, 1889), M. hapla (Chitwood, 1949) and M. thamesi (Chitwood, 1949) were, however, not attracted to their females (Santos, 1972) which tends to indicate that parthenogenetic females perhaps do not produce attractants.

Sex attraction has also been observed in animal-parasitic nematodes. Bonner & Etges (1967) noted that in Trichinella spiralis (Owen, 1835) males were attracted to females more strongly than females were to males. Similarly, Bone et al.. (1977)

observed sex attraction in Nippostrongylus brasiliensis (de Faria, 1910). Bone & Shorey (1977) further observed that one or more males in the presence of female attractants inhibited each other from responding. They (l.c.) also showed that attraction of males gradually decreased to increasing male:female ratios, but increased when the number of males was equal to or more than the females at the attractant source. More detailed studies of the reproductive behaviour of N. brasiliensis revealed that the maximum response of males coincided with the maturing age of the females (Bone et al., 1977). In addition, they noticed a decrease in the male response with age and a similar age related decrease in the pheromone production by the females. However, free-living nematodes generally became attractive to sex attractants after the final moult. But Cheng & Samoiloff (1971) reported that females of P. silusiae first produced attractants during the fourth juvenile stage and the males of the same stage were responsive. Duggal (1978a) failed to observe such a response in P. redivivus and proposed that the fourth stage juveniles used by Cheng & Samoiloff (1971) may have moulted during the experiment causing attraction of males. Cheng & Samoiloff (1972) associated production of sex attractants with gonad development. Disruption of the response of male N. brasiliensis to female pheromones was caused by pre-exposure of the males to female pheromones and, exposure to varying concentrations or for varying periods had almost identical effects (Bone &

Shorey, 1977). Such a change in the behaviour of the males was, however, reversible.

Despite the sizeable amount of work done on nematode sex attractants or pheromones, practically nothing is known about their chemical nature. Similarly, relatively little is known about the mechanism of chemosensory perception. Green (1966) while studying the orientation behaviour of males of G. rostochiensis and H. schachtii (Schm., 1871) to their females concluded that the males were attracted and retained by gustatory stimulation and Bird (1966) demonstrated the presence of esterases in the amphidial pouches of Meloidogyne javanica (Treub, 1885) and M. hapla and postulated that they might play a role in the sensory perception. McLaren (1976) suggested that amphids were multi-purpose receptors.

In the following work on reproductive behaviour three bisexual free-living soil nematode species were randomly selected viz., Chiloplacus symmetricus (Thorne, 1925), Curznema lambdiensis (Maupas, 1919) and Rhabditis sp. Although all the possible aspects could not be studied but, attempts have been made to study the mechanism of sex attraction, factors influencing sex attraction and the effect of age on sex attraction in C. symmetricus. In C. lambdiensis some of these aspects as well as a detailed study of the copulatory behaviour and some observations on the biological effects of ageing were carried out. The patterns of movement and the mechanism of orientation was studied in Rhabditis sp.

SEX ATTRACTION AND COPULATION IN *CHILOPLACUS SYMMETRICUS*

Since the observation of Greet (1964) that males and females of *Panagrolaimus rigidus* attracted each other, sex attraction has been described in a few other species. In some only the males were attracted to their females (Jones, 1966; Chin & Taylor, 1969; Azmi & Jairajpuri, 1977), while in others both sexes attracted each other (Cheng & Samoiloff, 1971; Balakanich & Samoiloff, 1974; Duggal, 1978a). There are conflicting reports as to when the sex attractants are produced. According to Cheng & Samoiloff (1971) the fourth-stage female juveniles of *Panagrellus silusiae* were capable of producing attractants and the fourth-stage male juveniles were capable of responding. However, Duggal (1978a) found no significant attraction in the fourth-stage juveniles of *P.redivivus*.

The present work was done to ascertain whether both sexes attracted each other or only the males responded and also to determine whether the fourth-stage juveniles were attractive or not. Besides, observations on orientation and copulation, of which not much is known in nematodes, were also carried out.

MATERIALS AND METHODS

Observations on sex attraction

Sex attraction was determined by the Petri dish method

(Fig. I). Twenty females, 1-5 days old were left for 24 hr in a Petri dish containing agar. Blocks taken from this dish served as the attractant source. The males were observed after 30, 60 and 90 min. There were 40 replicates. For controls, agar blocks were taken from Petri dishes containing no females.

Behaviour of males, females and juveniles to sex attractants:

To study the response of males, other females and fourth-stage male juveniles to female sex attractants, a 1 mm thick layer of 1 % water agar was poured into 15 cm diam Petri dishes. Three sectors were cut out leaving a triradiate structure with arms 1 cm broad and 7.5 cm long (Fig. 5a). An agar disc 1 cm in diam, containing secretions from twenty 1-5 day old females (virgin and non-virgin mixed) was placed at the junction of the three arms. Arcs concentric with the block were drawn so that each arm was further divided into seven equal sectors. 30 males, females and fourth-stage male juveniles were placed separately on each arm, 3.5 cm from the centre. The distribution of the nematodes were noted at hourly intervals for a period of four hours. There were three replicates.

A similar experiment was designed to see whether the males and fourth-stage female juveniles also emit sex attractants and whether females, males and fourth-stage male juveniles respond to

them (Fig. 5b). 1 cm diam agar blocks were taken from separate Petri dishes containing 1-5 day old males, 1-5 day old females and fourth-stage female juveniles. Each was placed in a separate arm 4 cm from the centre. 30 males, 30 females (of the same age as the ones to be tested) and 30 fourth-stage male juveniles were placed at the centres in separate Petri dishes and their distribution was recorded hourly for 4 hours. There were three replicates.

Orientation behaviour

To study the orientation behaviour of males to females, a 5 mm diam agar disc containing female secretions was put in the centre of a 7 cm diam Petri dish containing 1% agar. Males were released at various points and observed individually and the tracks they made were drawn with a camera lucida.

Copulation

Copulation was studied in observation chambers either under a stereoscopic binocular microscope or a compound microscope.

RESULTS

In the absence of female secretions, the distribution of the males in the three zones was nearly random (Fig. 3). They moved

about actively in large spiral or polygonal paths, freely entering or leaving the three zones. In the presence of female secretions, there was a persistent bias towards the central block (Fig. 3). If random, the distribution should correspond to ranks two or three. After half an hour, the distribution corresponds to ranks 1, 3, 4, 6, 7, 8, 9, 11, 12 and 13 but attraction is not evident (mean log score = 1.49; $P > 0.05$). After a lapse of 60 min however, the distribution corresponds to ranks 7,8, 12, 13 , 14 and 19 (mean log score = 2.25; $P < 0.001$) and another 30 min later to 12, 13, 14, 16, 17 and 19 (mean log score = 2.42; $P < 0.001$) indicating an increase in the attractiveness of the extract and a greater response by the males. By plotting the mean log scores of rank frequencies against time (Fig. 4) there was an increase in the attractiveness towards the central block. Differences in the mean log scores after 30 and 90 min were significant ($P < 0.001$).

The ability to perceive and respond to sex attractants is a characteristic which is exhibited by adult males only. After 4 hr, more than two-thirds of the males had accumulated at the central block (Fig. 6). Although their distribution was restricted to one plane, the gathering of the males at the female disc was significant (χ^2 , df = 21, $P < 0.001$). Juveniles moved unrestrictedly and distributed themselves randomly (χ^2 , df= 21, $P > 0.05$). A few reached the female disc by chance. Females

FIG. 3

Attraction of males to female secretions.

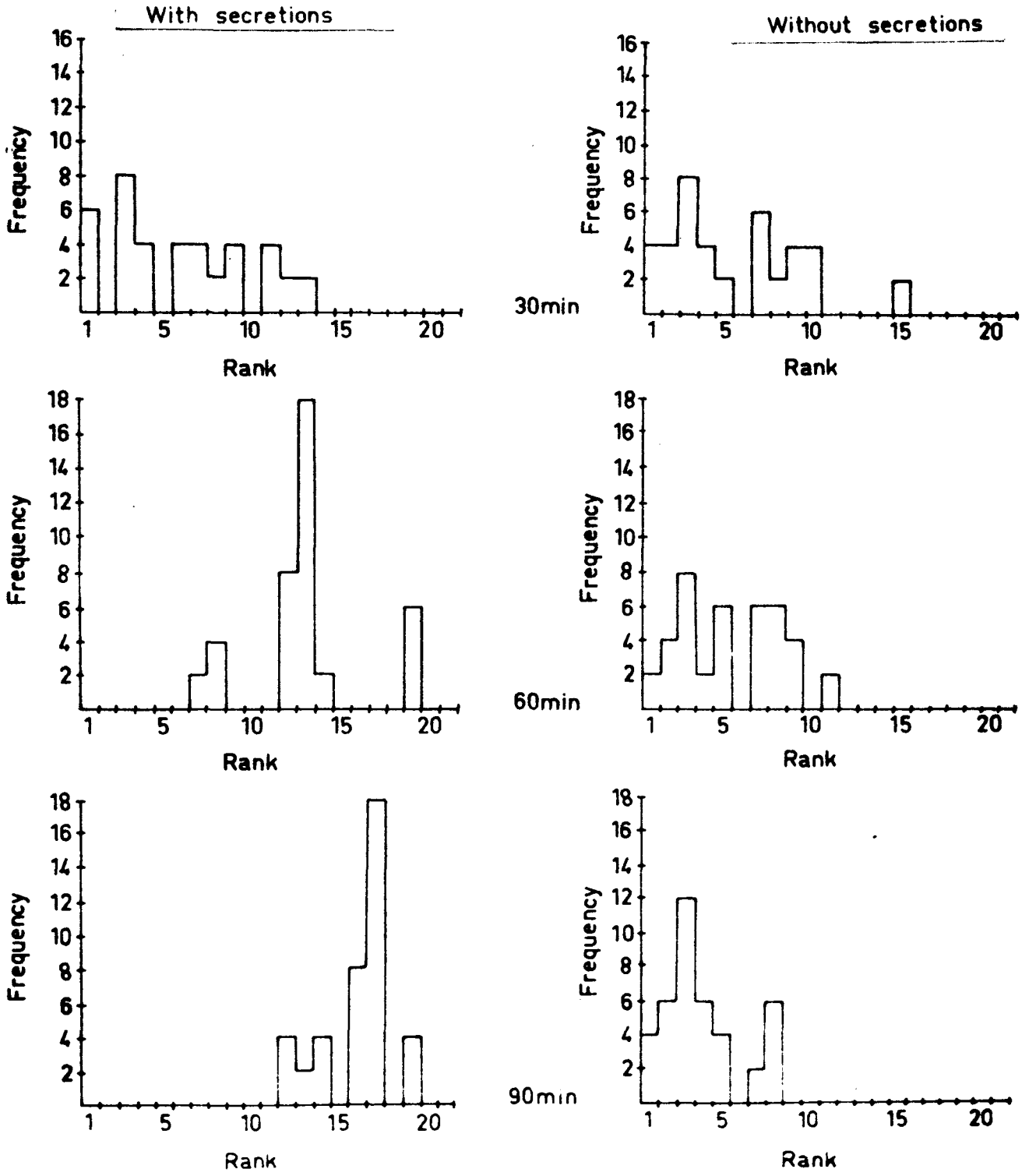

With secretions
Without secretions
Frequency
Rank
30min
60min
90min
1
5
10
15
20

FIG. 5

FIG. 4

Change of mean log score with time.

FIG. 5

a - Apparatus for testing attractiveness of males, females and juveniles to female secretions. I.P. = Inoculation point,

Att = Attractant.

b - Same for testing attractiveness of males, females and fourth-stage male juveniles to male, female and fourth-stage female juvenile secretions. I.P. = Inoculation point; M.S. = male secretions; F.S. = Female secretions; J.S.=Juvenile secretions.

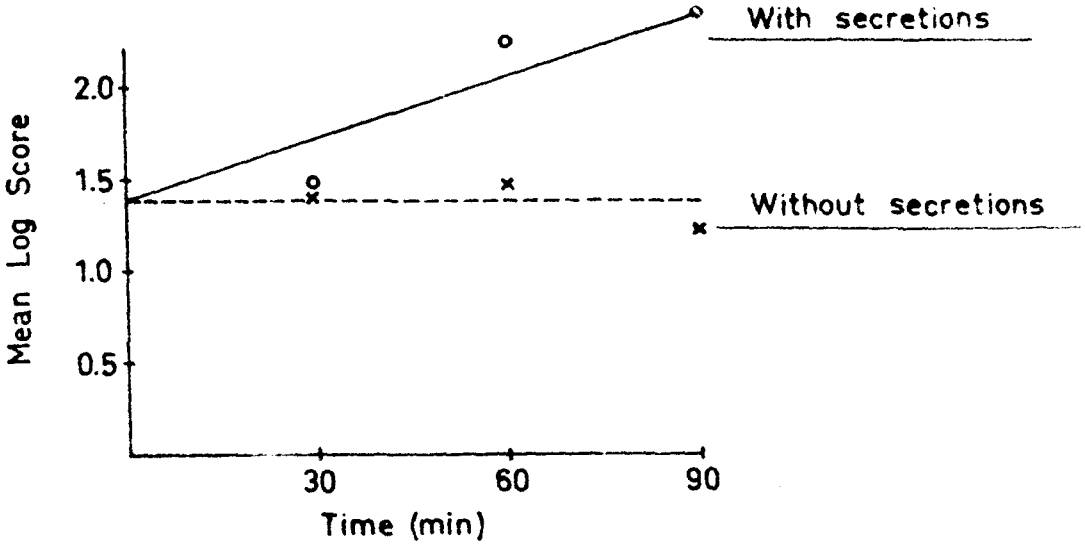

With secretions
Without secretions
Mean Log Score
2.0
1.5
1.0
0.5
30
60
90
Time (min)

FIG. 4

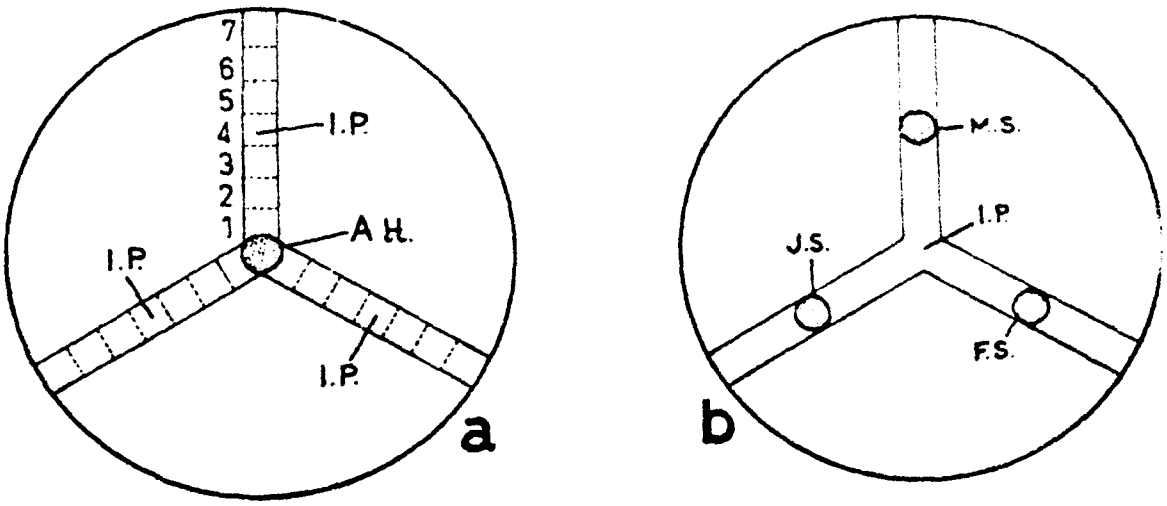

7
6
5
4
3
2
1
I.P.
A.H.
I.P.
I.P.
a
M.S.
I.P.
J.S.
F.S.
b

FIG. 5

moved little compared with males and juveniles. They were not significantly attracted towards the central block (χ^2, df= 21, $P \geq 0.1$).

Fourth-stage female juveniles failed to produce attractants (Fig. 7). Significantly more males moved into the arm containing the female extract than into those containing male or juvenile secretions ($P < 0.001$) reaffirming the presence of male attractants in female secretions. The females did not respond to any of the extracts and they rarely moved more than 3 cm from their starting point. Although there were females in the arm containing juvenile secretions than in those containing male or female, differences were insignificant ($P \geq 0.05$). The fourth-stage male juveniles though more active than the adult females, did not move in significant numbers towards any extract ($P \geq 0.05$), indeed their undirected movements frequently took them over the sides of the arms of the agar.

Tracks left on the surface of the agar helped interpretation of the behavioural response. Males on perceiving the stimulus displayed a characteristic turning which placed them on a path which might lead to the source (Fig. 8A). Movements towards the source was smooth with few lateral probings but with occasional lifting of the head above the agar surface. When orienting towards the source of attractant, the males never moved

FIG. 6

Distribution of males, females and fourth-stage male juveniles after 1, 2, 3 and 4 hr. attraction of males to females is significant (χ^2, df = 21, P= $<$0.001), females to females insignificant (χ^2, df= 21, P= $>$ 0.05) and fourth-stage male juveniles to females not significant (χ^2, df= 21, P= $>$ 0.05). Att = position of attractant.

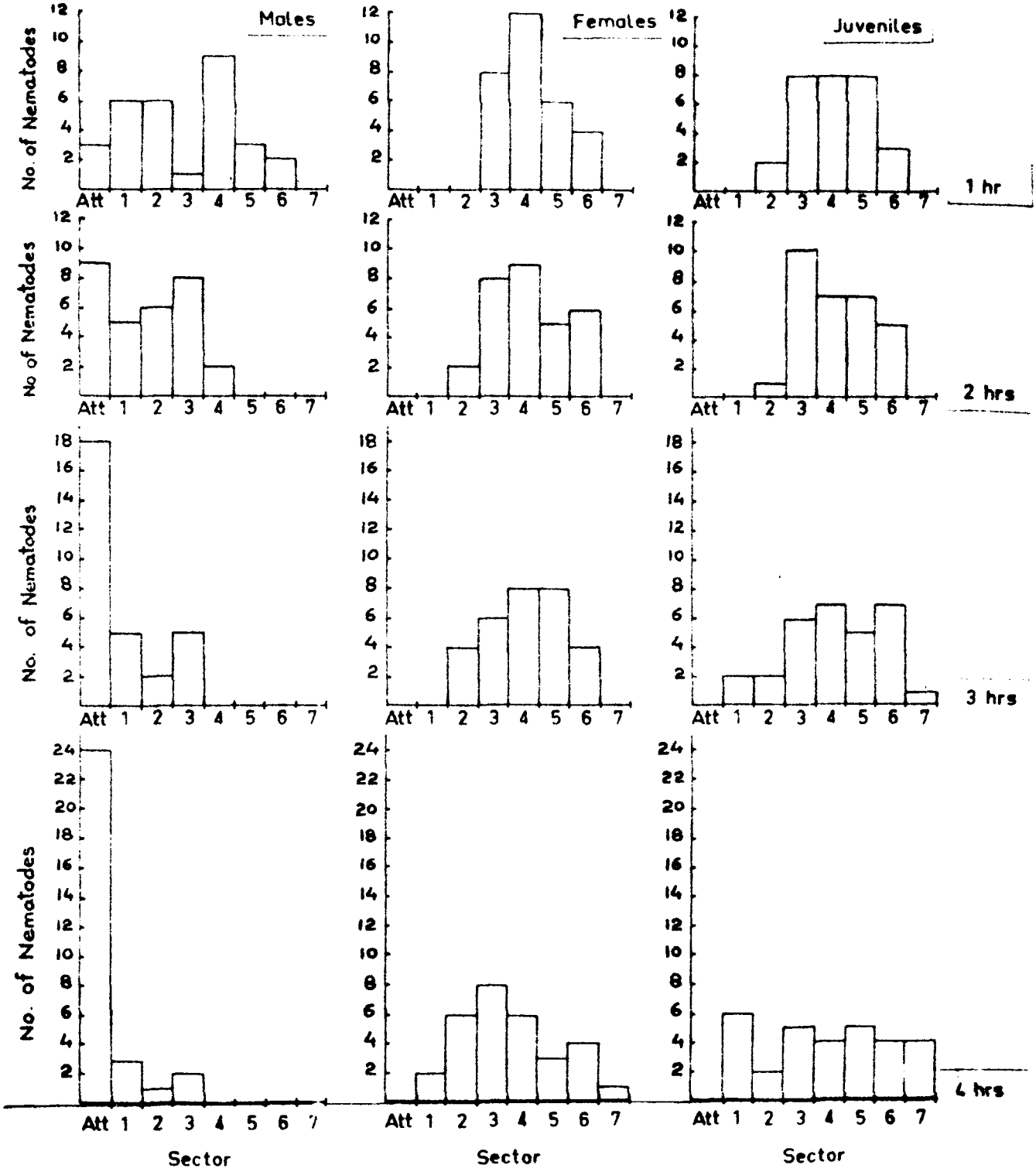

Males
Females
Juveniles
No. of Nematodes
1 hr
2 hrs
3 hrs
4 hrs
Att 1 2 3 4 5 6 7
Sector

FIG. 6

FIG. 7

Attraction of males, females and fourth-stage male juveniles to male, female and fourth-stage female juvenile secretions. Attraction of males to females significant ($P=<0.001$), females to fourth-stage female juveniles and fourth-stage male juveniles to females insignificant ($P=>0.05$).

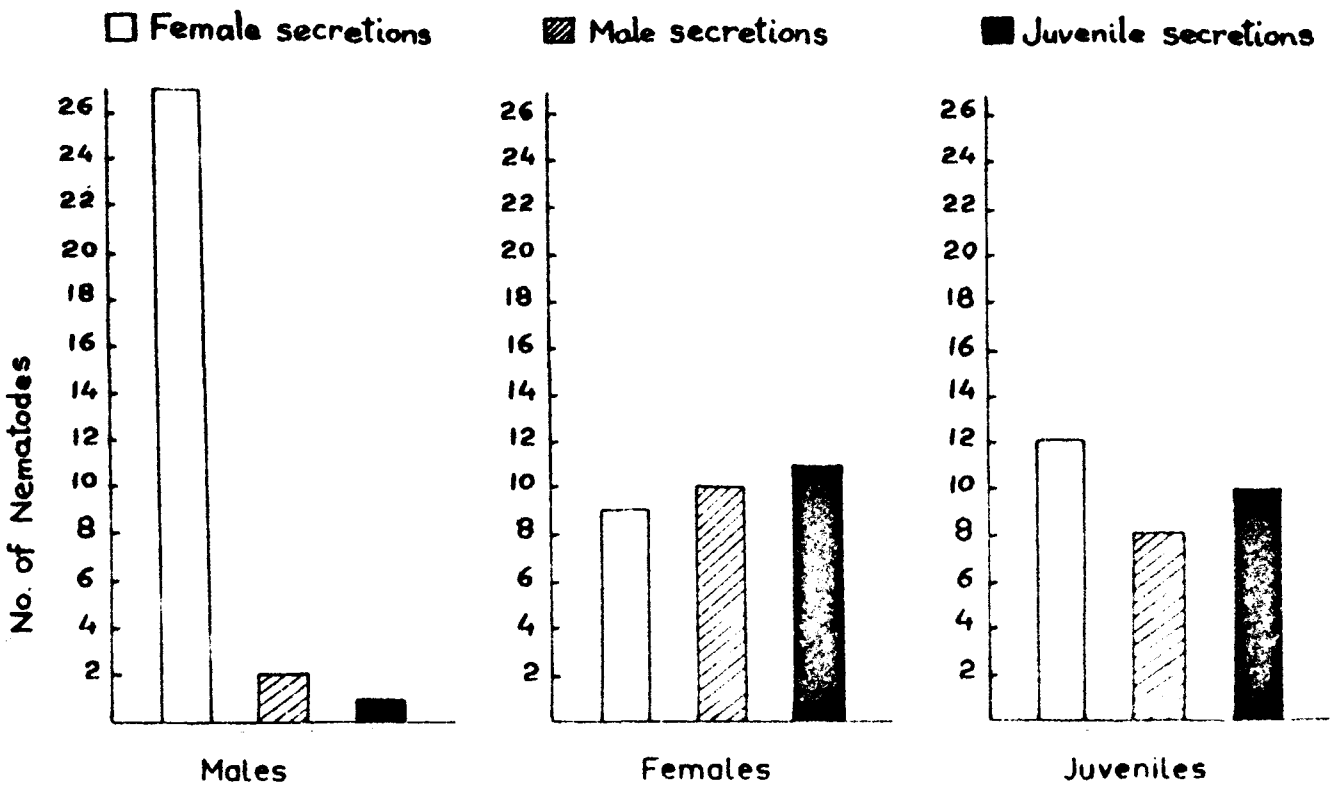

Female secretions
Male secretions
Juvenile secretions
No. of Nematodes
26
24
22
20
18
16
14
12
10
8
6
4
2
Males
Females
Juveniles

FIG. 7

along a direct path. Nearer the source there was a final readjustment marked by rapid turns and reversals after which they moved directly to their target. Occasionally an exaggerated response in the final turn put the male off its path. Then, after the male had moved for a short distance, there was an intense coiling of the body followed by sharp reversals. Thereafter, there was much lateral swinging of the head until it had again oriented to the attractant. Response of males may be divided into four stages. First, detection of the stimulus and orientation towards the attractant. Second, tangential movements with no apparent signs of lateral probings. Third, reorientation marked by much turnings and fourth, a direct orientation towards the stimulating source.

Copulation

In cultures, a tactile stimulus only may serve as a means of identifying another nematode in the neighbourhood. Males seemed unable to distinguish between the sexes and were frequently observed in a copulating posture with other males or juveniles.

When an active male came in contact with a female, it quickly orientated its body parallel to that of the female and typically moved its posterior third back and forth while closely appressed to that of the female. This probably aided in locating

FIG. 8

Tracks of males approaching female secretions. A - Typical approach path; B,C - Spiral polygonal approach. Arrows indicate starting points and S the source of attractant.

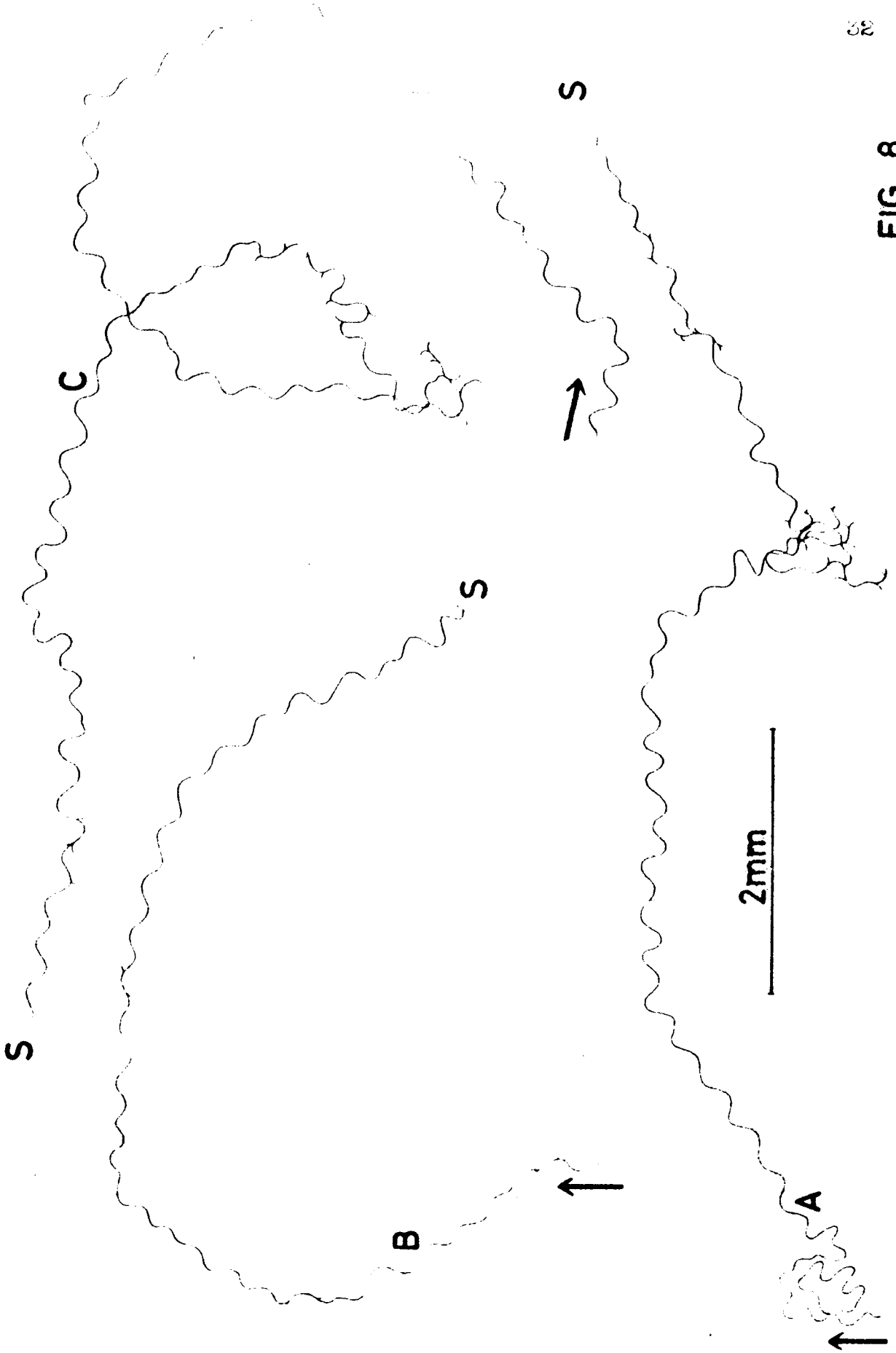


FIG. 8

the vulva for, soon afterwards the male coiled around the female, the ventrally flattened tail serving as an effective grasping surface. Within the coil, the female continued to move back and forth while the male probed with its spicules. Once the spicules had located the vulval opening, the female was firmly held. The spermatozoa were then transferred into the uterus. Although the actual movement of the sperm was not seen, a study of specimens fixed in formalin or stained in acetic orcein showed that after deposition in the uterus the spermatozoa moved upwards and accumulated in the spermatheca. A few travelled downwards and reached the post-uterine sac. Copulation lasted from half a min to nearly 45 min. During copulation both the sexes continued to feed actively.

DISCUSSION

Observations on the response of males of C. symmetricus to female secretions confirms the presence of male attractants which changes their behaviour and directs them towards maximum gradients. Male attracting substances occur not only in the secretions of immobile females such as Globodera rostochiensis and Heterodera schachtii (Green, 1966) but also in mobile females like those of Panagrolaimus rigidus (Greet, 1964), Pelodera teres (Jones, 1966). Cylindrocorpus longistoma and C. curzii (Chin &

Taylor, 1969), Acrobeloides sp. (Jairajpuri & Azmi, 1977), Hoplolaimus indicus (Azmi & Jairajpuri, 1977) and Panagrellus redivivus (Duggal, 1978a). Although females of C. symmetricus were not inactive, the male is the active partner in pairing.

Attraction of females to males was insignificant in Acrobeloides sp. (Jairajpuri & Azmi, 1977), but Greet (1964) observed aggregation of both the sexes of P. rigidus at cellophane barriers and Duggal (1978a) found virgin females of P. redivivus were attracted to adult males. Attractants in C. symmetricus evoke a response only in adult males and only the adult females emit sex attractants for the opposite sex. Fourth-stage female juveniles did not produce attractants or respond to them although fourth-stage female juveniles of Panagrellus silusiae produced attractants and the males of the same stage responded to them but copulation occurred only at maturity (Cheng & Samoiloff, 1971). Duggal (1978a) was unable to detect any attraction of adult males of Panagrellus redivivus to their fourth-stage female juveniles. However, he found that males were attracted to moulting and freshly moulted females and suggested that the fourth-stage juveniles used by Cheng & Samoiloff (1971) may have moulted during the experiment. Duggal (1978a) further showed that sex attraction in this species was dependant on the age and reproductive state of the worms.

Orientation of C. symmetricus males towards the source of attractant involved kinesis and taxes. The first turning response that put the male in the direction of the female is best considered klinokinetic; the subsequent tangential movement with few lateral probings but with occasional swinging of the head above the agar surface was probably klinotaxic. In view of the initial response and by reference to the definition of klinokinesis as the change in the intensity of turning due to change in the stimulus (Croll, 1971), the final reorientation near the source of the attractant may also be called klinokinetic as is the final direct approach. Fatigue of receptors to concentrations of attractants is not well demonstrated in this species, although males, after a normal approach occasionally 'get lost' when near the source, but they eventually reorientated and located the source.

The copulatory behaviour of C. symmetricus was similar to that of P. rigidus (Greet, 1964) and Cylindrocorpus sp. (Chin & Taylor, 1969), the only difference being that the male before coiling its tail around the female body moved back and forth with its tail appressed to the female. Duggal (1978b) showed in P. redivivus that the rate of copulation and the number of sperm transferred were related and the number of sperm transferred after 3 and 12 hr of isolation also differed. Long

periods of isolation produced a significant decline in the frequency of copulation but the number of oocytes fertilized did not change significantly. The copulatory posture, penetration and insemination were quite similar in P. redivivus and C. symmetricus.

FACTORS INFLUENCING SEX ATTRACTION IN *CHILOPLACUS SYMMETRICUS*

In various studies on sex attraction in nematodes, either live worms were used as the attractant source or only the female extracts left in the agar or the extracts of the culture media itself. A single female in *Globodera rostochiensis* or *Heterodera schachtii* could attract its males (Green, 1966) but a large number of individuals were required for a significant attraction in some other species such as *Panagrellus redivivus* (Duggal, 1978a). Further, the time required by the females to produce a suitable attractant gradient and the time needed by the males to respond to it is also quite variable. The following work was done in order to obtain a relationship, if any, between the number of worms at the attractant source and the response of males, and the duration of incubation of the worms producing the attractant and the male response. Besides, the time after which the males should be observed and the role of the agar media in influencing sex attraction was also studied.

MATERIALS AND METHODS

Tests on sex attraction were done by two methods, the Petri dish method and the mickey mouse trap. In all experiments

both the techniques were used except when testing the effect of thickness of agar on sex attraction. The Petri dish experiments were replicated 20 times and the mickey mouse experiments five times. The following tests were carried out.

Attraction to varying number of females: Attraction was tested in the Petri dish using 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 and 20 females. The females were kept in the straw pipe containing agar in the centre of the Petri dish for 24 hr before the males were released. The final distribution of the males was recorded after 4 hr. In the mickey mouse trap, 1, 10, 50 and 100 females were used to produce attractants. The incubation time was 24 hr and the final distribution of the males was recorded 4 hr after their release.

Duration of the incubation period: Females were incubated for periods varying from 6, 12, 18 and 24 hr before the males were released and their distribution was observed after 4 hr. In the Petri dish experiment one and five females were used while in the mickey mouse trap 10 and 50 females were used to produce attractants.

Time of observation of males: For the Petri dish experiment, 1, 5, 10 and 20 females were incubated for 24 hr

after which males were released and observed after 1, 2, 3,4 and 5 hr. In the mickey mouse trap 1, 10, 50 and 100 females were used and the males were observed similarly.

Thickness of the agar: In this experiment, Petri dishes with the following thickness of agar were used: 1, 2, 4 and 8 mm. There were two sets of experiments using one and five females.

Concentration of the agar: In this experiment, the concentration of the agar medium varied from 1, 2, 4 and 8%. One and five females were used to produce attractants in the Petri dish experiments and 10 and 50 females in the mickey mouse trap. In both the cases, the females were incubated for 24 hr and the males were observed after 4 hr.

Effect of light: To study the effect of light, one set of experiment was set up in diffused light and the other one in total darkness. Five females were used in the Petri dish experiment and 50 in the mickey mouse trap.

RESULTS

Attraction to varying number of females: Single females were capable of evoking a very mild response from the males (Fig. 9) in the Petri dish experiment (mean log score = 1.75;

FIG. 9

The effect of number of females on the attraction of males in the Petri dish experiment.

FIG. 10

The effect of number of females on the attraction of males in the mickey mouse trap.

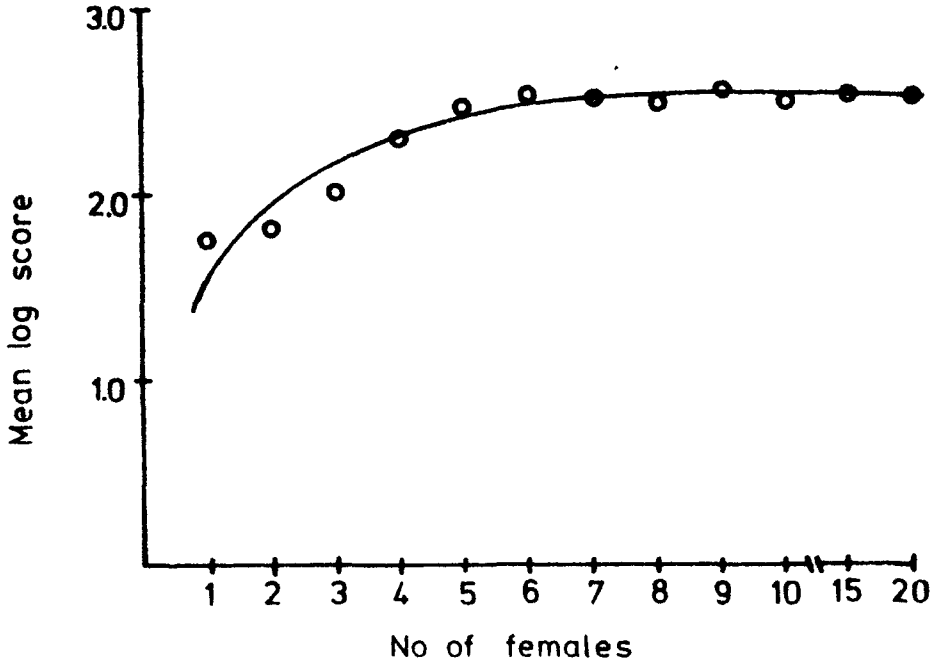

3.0
2.0
1.0
Mean log score
1 2 3 4 5 6 7 8 9 10 15 20
No of females

FIG. 9

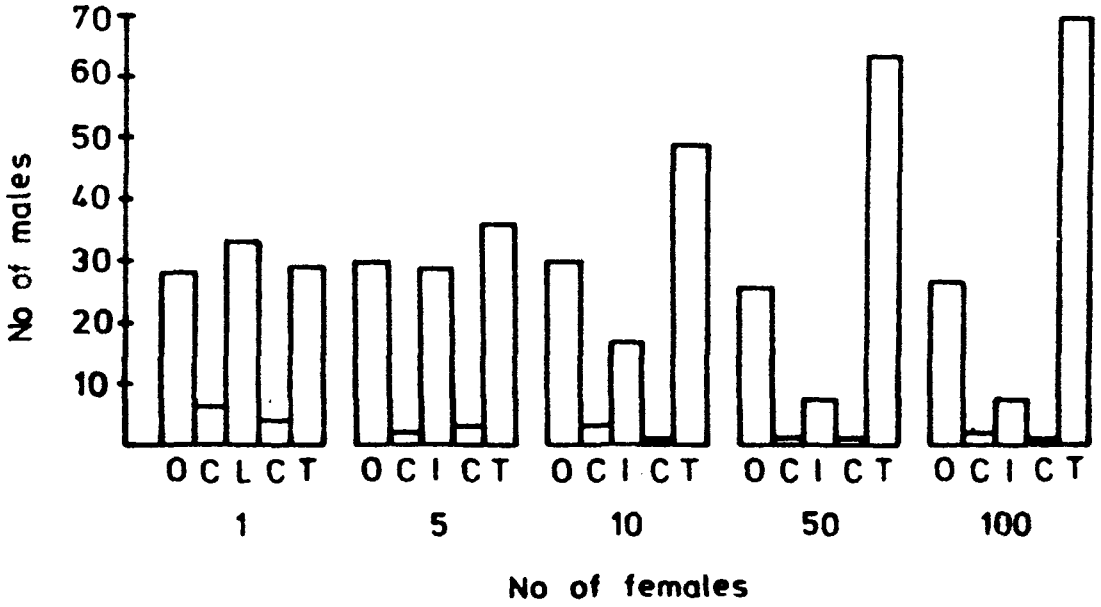

70
60
50
40
30
20
10
No of males
O C L C T O C I C T O C I C T O C I C T O C I C T
1 5 10 50 100
No of females

FIG. 10

$P = \langle 0.05$). With the increase in the number of females, attraction also increased correspondingly till the number of females was 5-7 (mean log score = 2.4; $P = \langle 0.001$). After this number, no parallel increase in the response of males was observed. It remained somewhat constant with the increase in the number of females and produced no significant increase in attraction ($P = \rangle 0.1$). In the mickey mouse trap (Fig. 10) single females evoked no response ($P = \rangle 0.1$) and at least ten were necessary for a positive response ($P = \langle 0.05$). Maximum attraction occurred when 50 or 100 females were present, there being no significant difference in the responses in the two cases ($P = \rangle 0.05$).

Duration of incubation of the females: Single females incubated for less than 12 hr in Petri dish experiments (Fig. 11) failed to enhance the response of the males (mean log score = 0.9). After 18 hr incubation the mean log score increased to 1.57 but attraction was not significant ($P = \rangle 0.05$). Only after 24 hr a significant response was observed (mean log score = 1.7; $P = \langle 0.01$). When five females were used males showed no significant response after 6 hr incubation (mean log score = 1.01; $P = \rangle 0.1$) but did so after 12 hr (mean log score = 1.73; $P = \langle 0.05$). Maximum attraction was observed after 18 or 24 hr incubation but the two were not significantly different between themselves ($P = \rangle 0.05$).

FIG. 11

The effect of varying incubation periods of females on the attraction of males in the Petri dish experiment.

FIG. 12

The effect of varying incubation periods of females on the attraction of males in the mickey mouse trap.

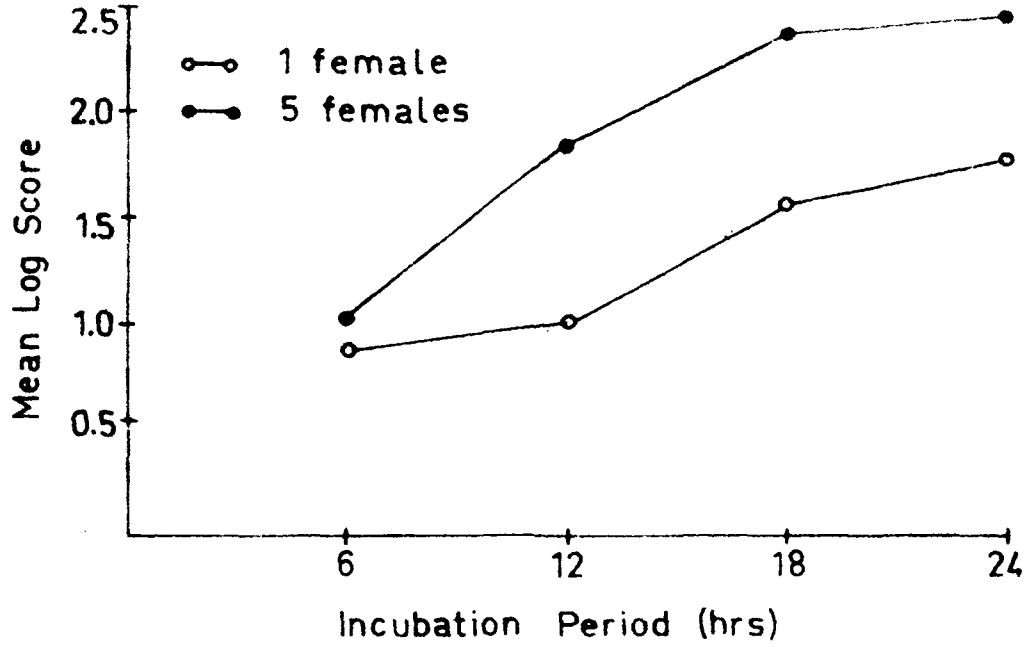

2.5
2.0
1.5
1.0
0.5
Mean Log Score
1 female
5 females
6
12
18
24
Incubation Period (hrs)

FIG. 11

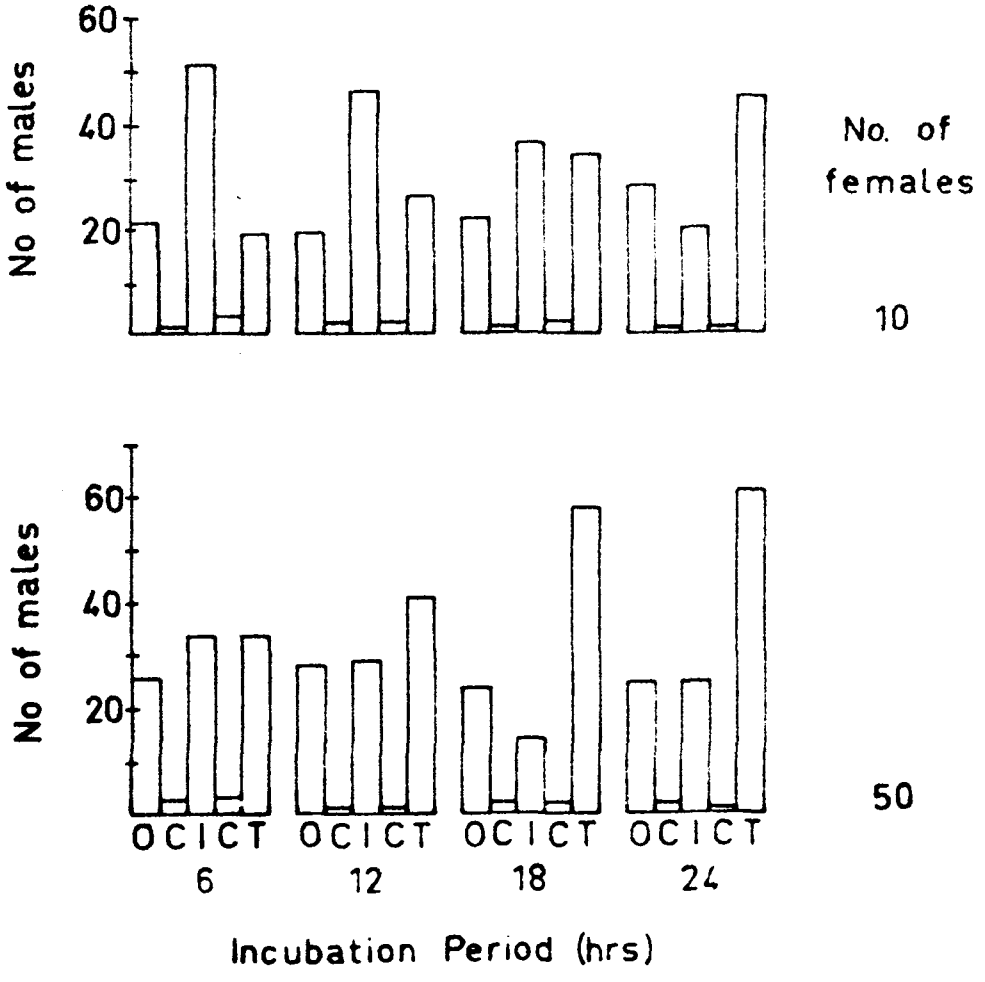

60
40
20
No of males
No. of females
10
50
O C I C T
6
12
18
24
Incubation Period (hrs)

FIG. 12

Incubation of females for less then 6 hr failed to attract males ($P=\rangle 0.1$) in the mickey mouse trap (Fig. 12). After 12 hr males did show a slight response to 50 females ($P=\langle 0.05$) but were still not attracted to 10 females ($P=\rangle 0.1$) At least 18 hr incubation was necessary when using 10 females to make males respond ($P=\langle 0.025$), but maximum attraction was observed after 24 hr incubation ($P=\langle 0.01$). Maximum attraction when using 50 females was also observed after 24 hr incubation but the number of males migrating to the test chamber after 18 hr was not significantly different from that after 24 hr ($P=\rangle 0.1$).

Time of observation of males: Males showed significant attraction towards single females in the Petri dish experiment (Fig. 13) 4 hr after their release ($P=\langle 0.05$) and towards five females after 2 hr ($P=\langle 0.025$). Attraction was evident within 1 hr when 10 or 20 females were used ($P=\langle 0.005$). Maximum attraction was observed after 3 hr with 5 females and after 2 hr with 10 or 20 females.

In the mickey mouse trap (Fig. 14), single females did not evoke a response from the males even after 4 hr ($P=\rangle 0.1$). When 10 females were used, attraction was evident after 3 hr and with 50 or 100 females after 2 hr ($P=\langle 0.025$). Maximum migration of males into the test chamber was observed after 4 hr in all the cases. The number of males migrating to the test chamber when

FIG. 13

Changes in the response of males with increase in their observation time in the Petri dish experiment.

FIG. 14

Changes in the response of males with increase in their observation time in the mickey mouse trap.

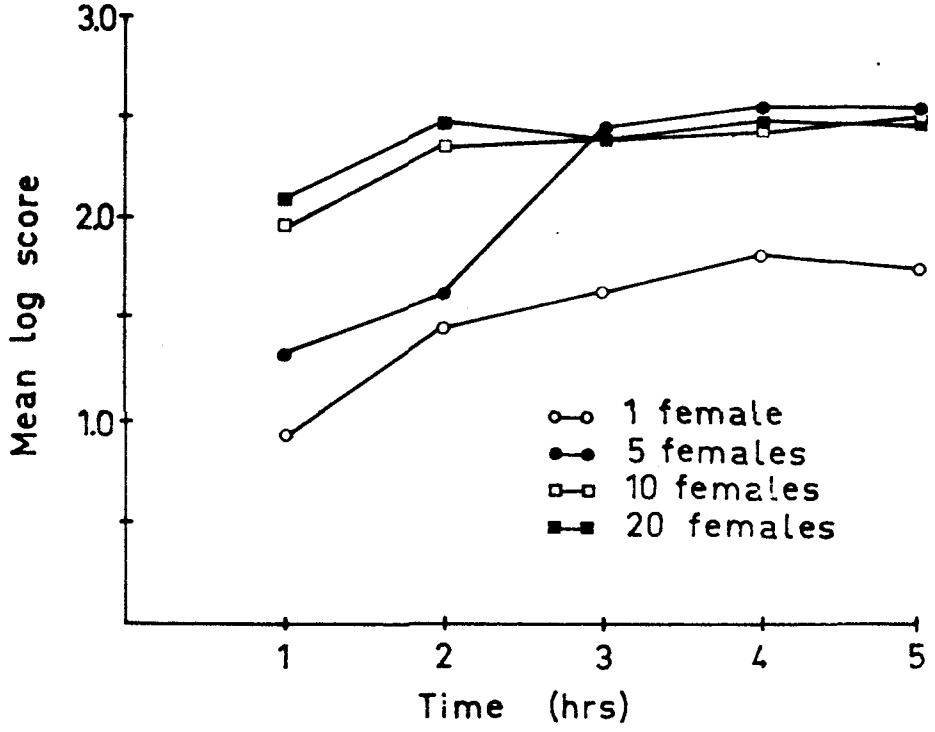

3.0
2.0
1.0
Mean log score
1 female
5 females
10 females
20 females
1
2
3
4
5
Time (hrs)

FIG. 13

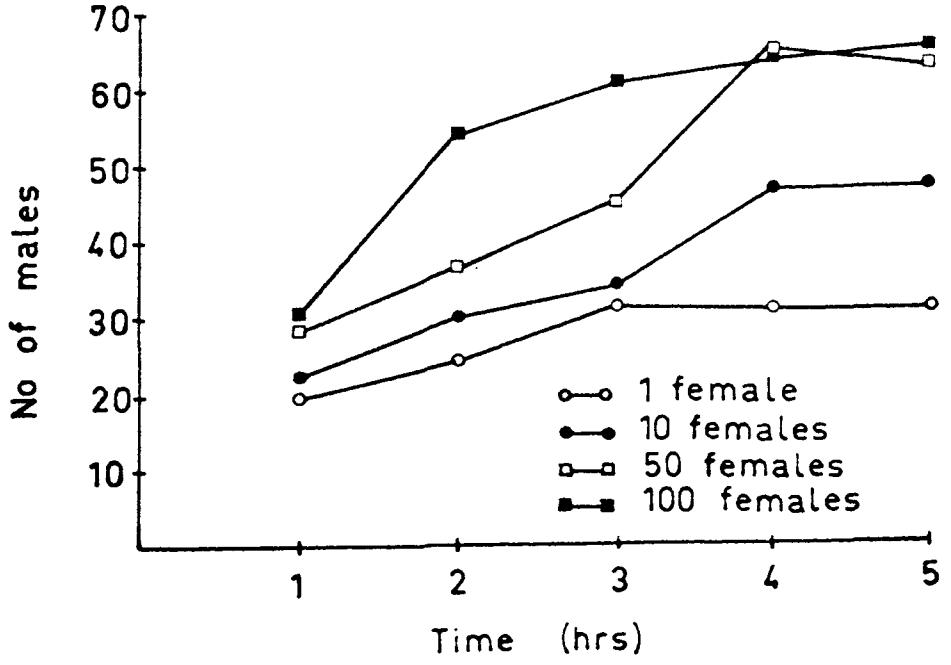

70
60
50
40
30
20
10
No of males
1 female
10 females
50 females
100 females
1
2
3
4
5
Time (hrs)

FIG. 14

using 100 females was not significantly different after 3 or 4 hr ($P \geq 0.1$).

Thickness of the agar: (Fig. 15 A) Agar thickness of 1, 2 and 4 mm produced no significant differences in the attraction of males when one and five females were used ($P > 0.05$), but at 8 mm thickness the males did not respond significantly to single females ($P \geq 0.1$). Attraction to five females also decreased significantly ($P < 0.01$) at this thickness but the response remained positive (mean log score = 1.70).

Concentration of the agar: (Fig. 15 B, C) 4 and 8% agar inhibited sex attraction in both Petri dish and mickey mouse experiments. 1 and 2% agar seemed optimum for there was no significant difference in the attraction of males in these two media ($P > 0.05$).

Effect of light: Differences in the attraction of males to females in diffused daylight and in total darkness were not significant ($P > 0.1$) in either the Petri dish experiment or the mickey mouse trap.

DISCUSSION

Sex attraction in Chiloplacus symmetricus was dependant on the concentration of the attractant in the medium as well as

FIG. 15

A - The effect of thickness of agar on the response of males in the Petri dish experiment. o—o one female; ●—● five females.

B - The effect of concentration of agar on the response of males in the Petri dish experiment. o—o one female; ●—● five females.

C - The effect of concentration of agar on the response of males in the mickey mouse trap.

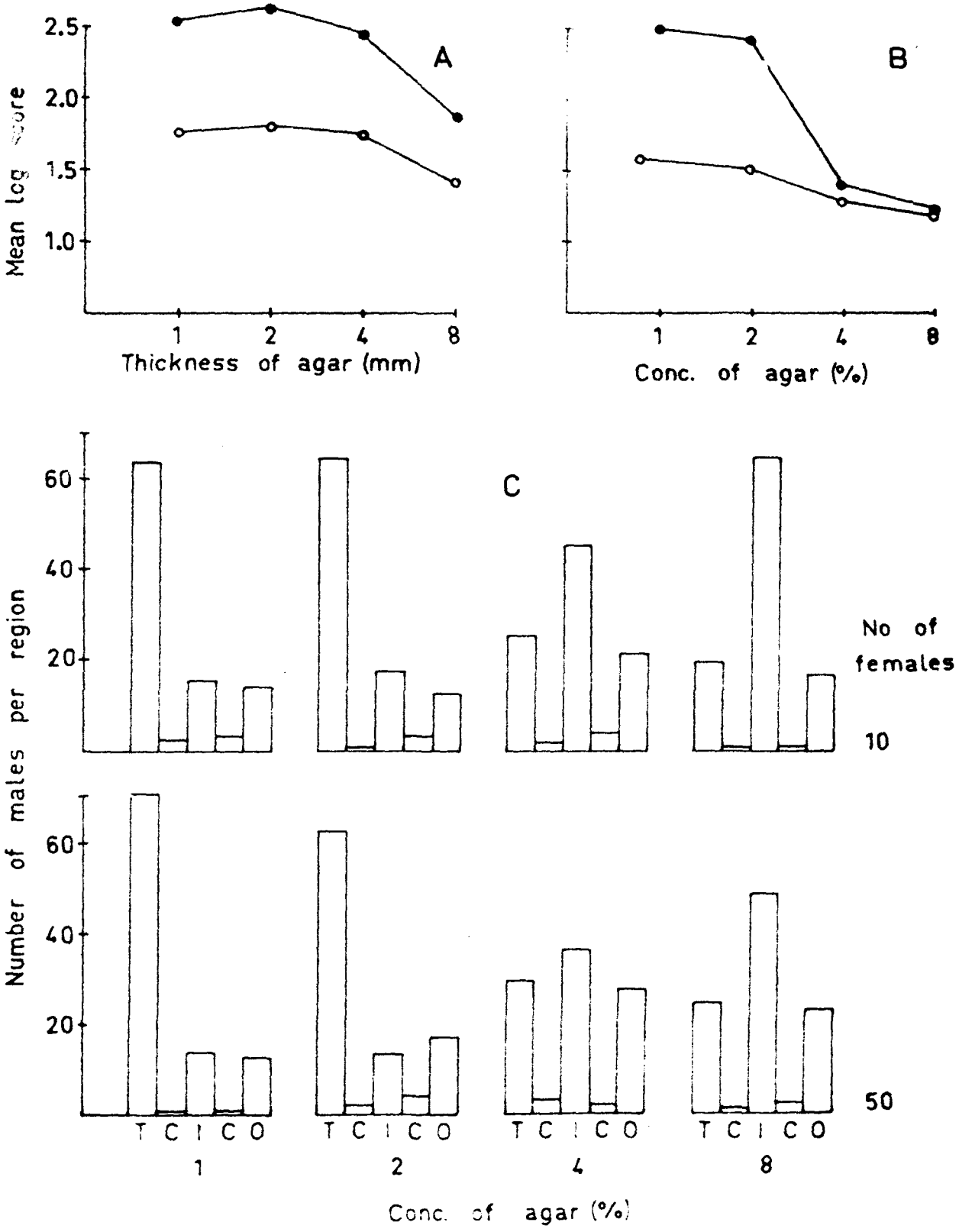

Mean log score
2.5
2.0
1.5
1.0
A
1
2
4
8
Thickness of agar (mm)
B
Conc. of agar (%)
Number of males per region
60
40
20
C
No of females
10
50
T C I C O
Conc. of agar (%)

FIG. 15

the time given to the males to respond. Both these factors varied with the experimental techniques employed and were between themselves directly related, i.e., the greater the concentration of the attractants, the more quickly the males responded. The responses of the males under these two factors were, however, limited and once the optimum concentration was attained, attraction ceased to increase (Fig. 9). In mickey mouse chambers, hardly more than 65% of the males showed a positive response, but in the Petri dish experiments as many as 80% (corresponding to rank scores 18 and 19) of the males often reached the plastic straw pipe containing the females attractants. Besides the smaller number of males responding, more females were required to produce a significant attraction in the former experiment than in the latter. Such differences in attraction probably arose from basic orientation drawbacks encountered by males in the mickey mouse chambers as well as weaker attractant resulting from diffusion through the narrow connecting channels. Detection of the attraction stimulus in C. symmetricus has been attributed to klinokinesis and as such, the chances of males coming across attractants were far more in the Petri dish experiments where movement is unrestricted, than in mickey mouse chambers. Thus it is concluded that the weaker gradients in the mickey mouse chambers caused a weaker response of the males.

The period of incubation and attraction also seem to be related in the same way as the number of males and attraction. The optimum concentration of attractants occurred after 18 hr incubation when 50 females were used in the mickey mouse chambers and five females in the Petri dish experiments. This is in contrast to Globodera rostochiensis and Heterodera schachtii where only 2.5 hr incubation developed a good gradient (Greet et al., 1968) but somewhat similar to Panagrellus redivivus (Duggal, 1978a) and Cylindrocorpus longistoma and C. curzii (Chin & Taylor, 1969).

While a decrease in attraction of males in high concentrations of agar is conceivable primarily due to inhibition of locomotion on a stiffer surface, the apparent insignificant difference in attraction to different thickness of agar is somewhat perplexing in view of the fact that attractants would have to diffuse over a large volume of media in thicker layers. However, it is probably the diffusion characteristics of the attractants in different agar media which perhaps plays the key role in this case. Hence, sex attraction will definitely vary with the experimental conditions in different nematode species and it is suggested that before experimentation, the conditions ideal for attraction should be evaluated.

AGEING AND SEX ATTRACTION IN CHILOPLACUS SYMMETRICUS

Ageing in different nematode species has been found to produce both morphological and biochemical changes (Gershon & Gershon, 1970; Zuckerman et al., 1973; Hogger et al., 1977) which ultimately manifest themselves by modifying biological activities and possibly also effecting behavioural characteristics. The reproductive period of the vinegar eelworm Turbatrix aceti decreased when ageing females were mated with young females or conversely, when young males were mated with ageing females (Kisiel & Zuckerman, 1974) and similarly Duggal (1978a) reported a decrease in the attraction of males to ageing virgin females of Panagrellus redivivus.

In the present work an attempt has been made to study the sex attraction behaviour of virgin as well as non-vigin males and females of Chiloplacus symmetricus.

MATERIALS AND METHODS

Chiloplacus symmetricus was reared in 5.5 cm Petri dishes in malt-peptone agar. Synchronous cultures were initiated by sieving 3-5 day old cultures in a modified Baermann's funnel

and collecting the nematodes after 36 hr. This suspension usually contained nematodes of all stages. After this the funnel was reset and left for another 24 hr. At the end of this period, mostly freshly hatched juveniles were obtained which were transferred to culture media. Virgin males and females were obtained by separating the sexes during the fourth moulting stage when they were easily distinguishable and were maintained in separate Petri dishes. Non-virgin males and females were obtained by allowing the sexes of the same age to grow together in Petri dishes, 25 pairs per dish. They were transferred to fresh media every second day to avoid overlapping of generations.

Sex attraction was tested by the mickey mouse trap method (Fig. 2). Males and females were divided into four age groups viz., 10, 14, 18 and 22 day old. Each age group of males was tested against each age group of female i.e., 10 day old males were tested with 10, 14, 18 and 22 day old females; 14 day old males with 10, 14, 18 and 22 day old females and so on. Sex attraction of the various age groups was determined between virgin males and virgin females. virgin males and non-virgin females, non-virgin males and virgin females and between non-virgin males and non-virgin females.

RESULTS

Attraction between virgin males and virgin females: (Fig. 16)

All age groups of males showed significant attraction towards 10, 14 and 18 day old females ($P = < 0.025$). While 10 and 14 day old males were also attracted to 22 day old females ($P = < 0.01$), 18 and 22 day old males showed no significant attraction ($P = > 0.1$).

Responses of ageing males to 10 day old females: Although ageing males showed a significant response to 10 day old females, differences between 10 and 18, and 10 and 22 day old males were significant ($P = < 0.025$; $P = < 0.01$ respectively). Similarly, differences between 14 and 22 day old males was significant ($P = < 0.025$). Differences between the other age groups of males was not significant ($P = > 0.1$).

Responses of ageing males to 14 day old females: 10 and 14, and 10 and 18 day old males showed no significant difference ($P = > 0.1$) but the difference between 10 and 22 was significant ($P = < 0.05$). The responses of 14 and 22 day old males differed significantly ($P = < 0.001$) but between 14 and 18, and 18 and 22 showed no difference ($P = > 0.1$).

Responses of ageing males to 18 day old females: None of the age groups of males showed significant difference among themselves.

FIG. 16

The effect of male and female age on the attraction of virgin males to virgin females.

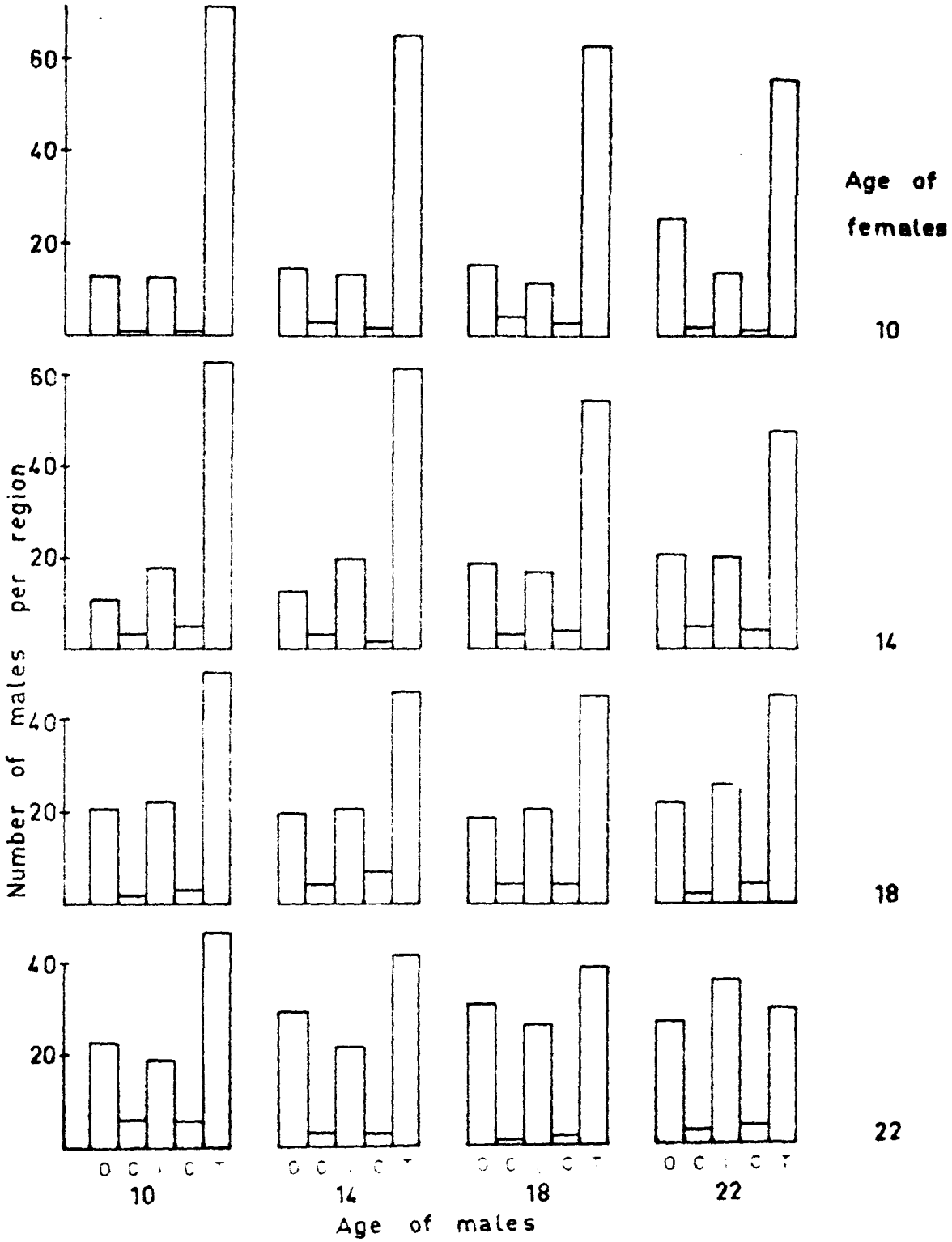

Number of males per region
60
40
20
Age of females
10
14
18
22
O C I C T
Age of males

FIG. 1o

Responses of ageing males to 22 day old females: There was no significant difference in the response of 10 and 14 day old males ($P = > 0.1$). Differences between other age groups was not calculated due to lack of attraction in any one of the compared groups.

Responses of 10 day old males to ageing females: Differences in the male response to 10 and 14 ($P = < 0.05$), 10 and 18 ($P = < 0.005$) and 10 and 22 day old females ($P = < 0.001$) were significant. Similarly, between 14 and 18 ($P = < 0.025$) and 14 and 22 ($P = < 0.005$) day old females were also significant, but between 18 and 22 was not significant ($P = > 0.1$).

Responses of 14 day old males to ageing females: Male responses to 10 and 14 day old females was not significantly different ($P = > 0.05$). However, differences in response to 10 and 18 ($P = < 0.005$) and 10 and 22 ($P = < 0.001$) day old females were significant. Similarly, differences between 14 and 18 ($P = < 0.01$) and 14 and 22 ($P = < 0.001$) were also significant but between 18 and 22 not significant ($P = > 0.05$).

Responses of 18 day old males to ageing females: Attraction of males differed significantly between 10 and 18 ($P = < 0.001$) and 10 and 22 ($P = < 0.001$) day old females and also between 14 and 18 day old females ($P = < 0.001$), but not between 10 and 14 day old

females ($P=>0.1$). Differences between other groups was not tested because there was no significant attraction in one or both the groups.

Responses of 22 day old males to ageing females: Differences between 10 and 14, and 10 and 18 day old females were significant ($P=<0.025$; $P=<0.01$ respectively) but between 14 and 18 was not significant ($P=>0.05$). Differences between other groups was not calculated because of lack of attraction.

Attraction between virgin males and non-virgin females: (Fig. 17)

Only 10 day old non-virgin females were attractive to all age groups of males. 22 day old males were not attracted to 14 day old females ($P=>0.05$) but 10, 14 and 18 day old males showed a significant response ($P=<0.01$). None of the age groups of males were attracted to 18 and 22 day old females ($P=>0.1$).

Responses of ageing males to 10 day old females: The differences in responses of 10 and 14, and 10 and 18 day old males were not significant ($P=>0.1$) but between 10 and 22 was significant ($P=<0.025$). 14 and 18 day old male responses differed insignificantly ($P=>0.1$) and similarly, differences between 14 and 22, and 18 and 22 day old males were not significant ($P=>0.05$).

FIG. 17

The effect of male and female age on the attraction of virgin males to non-virgin females.

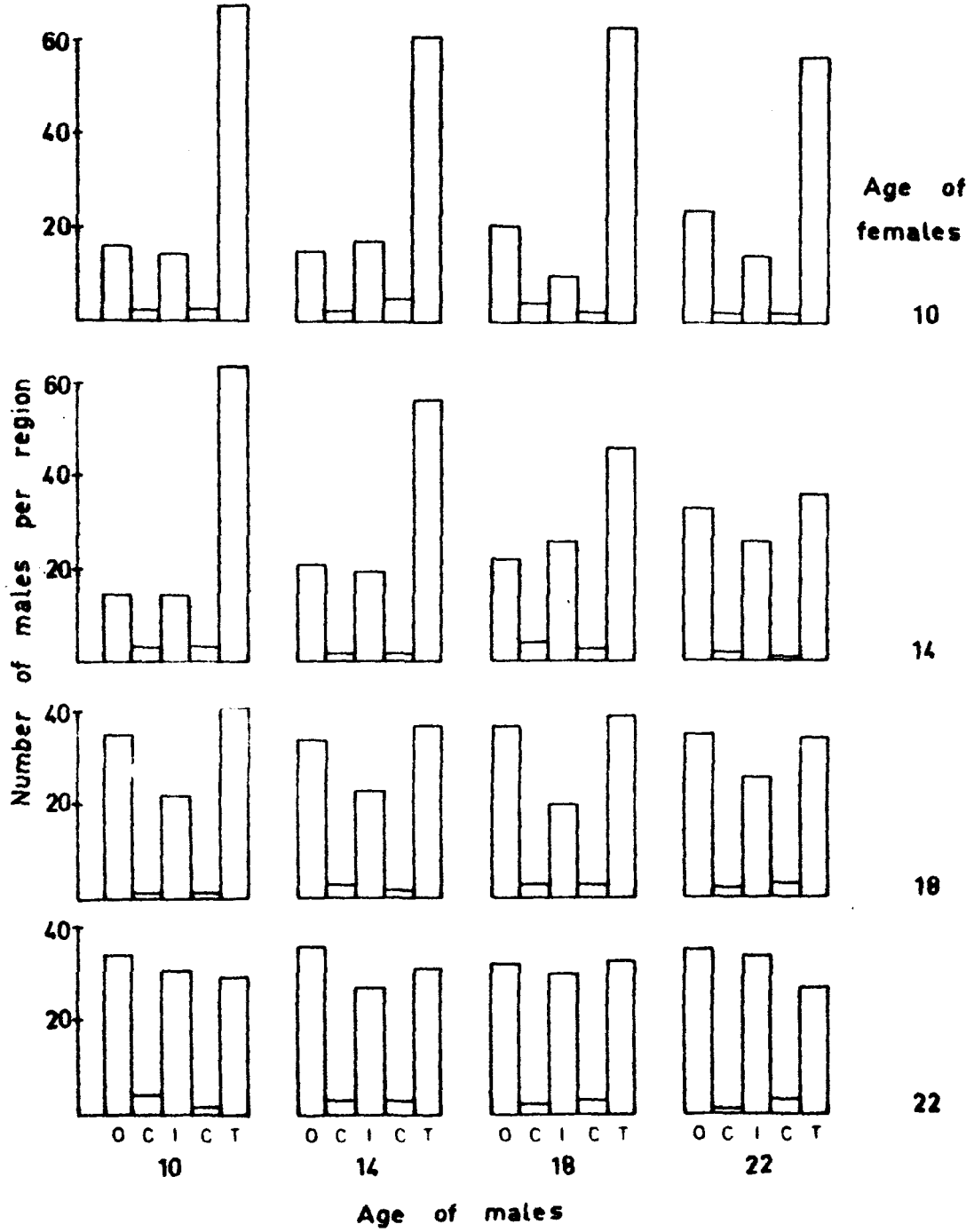

Number of males per region
60
40
20
40
20
40
20
O C I C T
10
O C I C T
14
O C I C T
18
O C I C T
22
Age of males
Age of females
10
14
18
22

FIG. 17

Responses of ageing males to 14 day old females: The difference only between 10 and 14, 10 and 18, and 10 and 22 day old males were calculated. Of these, the first one was not significant ($P = > 0.05$) but the second and third were significant ($P = < 0.005$ and $P = < 0.01$ respectively).

Differences in responses of ageing males to 18 and 22 day old females were not evaluated because of lack of attraction.

Responses of males to ageing females: Since 18 and 22 day old females were unattractive ($P = > 0.05$) differences in the responses of males to ageing females could only be calculated between 10 and 14 day old females. The responses of 10 day old males to 10 and 14 day old females was insignificant ($P = > 0.1$). Similarly, the response of 14 day old males to 10 and 14 day old females was not significant ($P = > 0.1$). However, the difference in the response of 18 day old males to 10 and 14 day old females was significant ($P = < 0.005$).

Attraction between non-virgin males and virgin females: (Fig. 18)

All age groups of males showed significant attraction ($P = < 0.01$) towards ageing females except 22 day old males to 18 and 22 day old females ($P = > 0.05$).

Responses of ageing males to 10 day old females: Comparisons of responses to 10 and 14, and 10 and 18 day old males were not

FIG. 18

The effect of male and female age on the attraction of non-virgin males to virgin females.

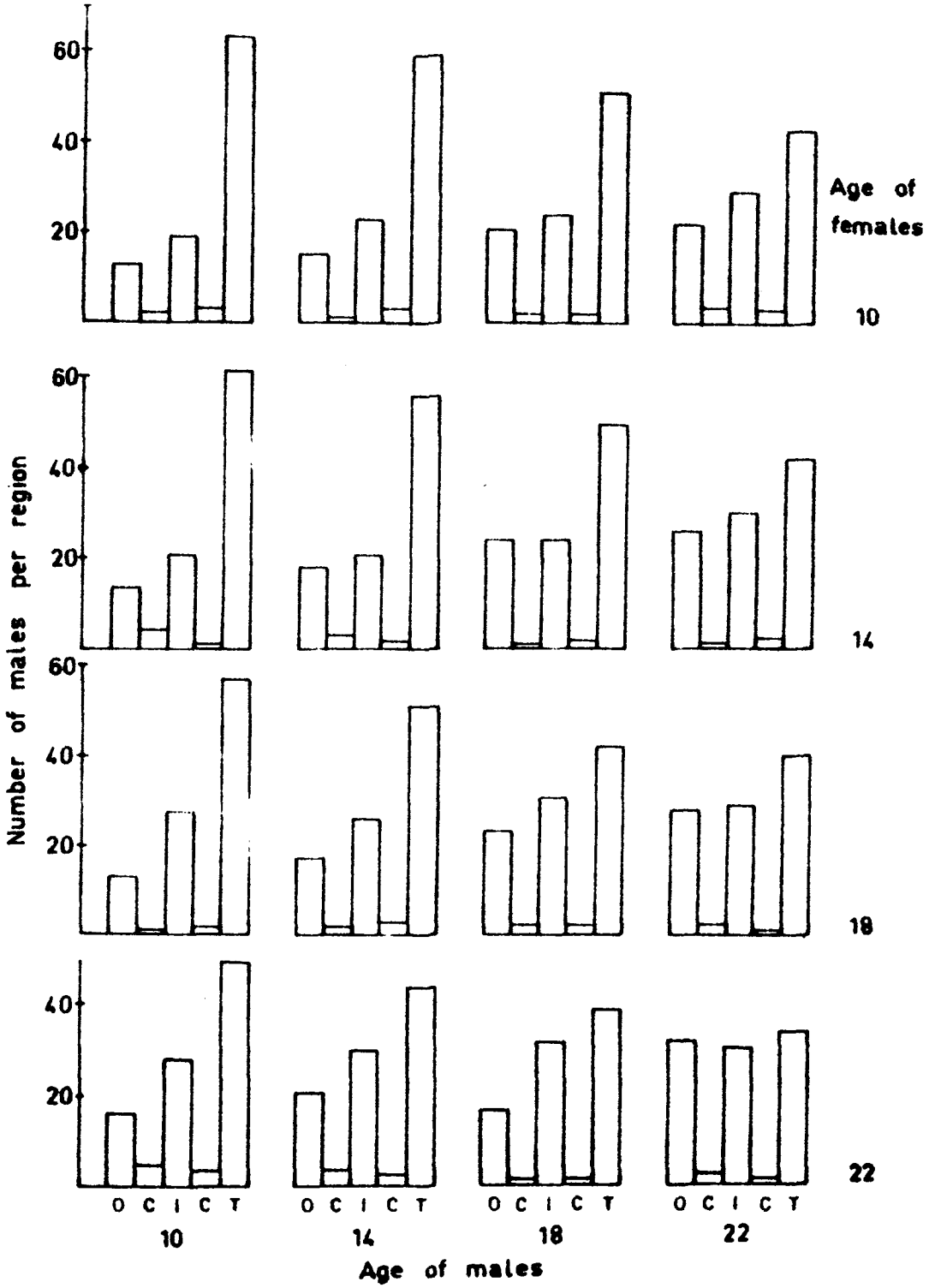

Number of males per region
60
40
20
Age of females
10
14
18
22
O C I C T
Age of males

FIG. 18

significant ($P=>0.1$) and between 10 and 22 was significant ($P=<0.001$). Similarly, the differences in the response of 14 and 18, and 18 and 22 day old males were not significant ($P=>0.1$) but between 14 and 22 was significant ($P=<0.01$).

Responses of ageing males to 14 day old females: Differences only between 10 and 14 day old males were not significant ($P=>0.1$) while the differences in the responses of 10 and 18 ($P=<0.05$), 10 and 22 ($P=<0.001$) and 14 and 22 ($P=<0.005$) day old males were significant. Differences between 14 and 18, and 18 and 22 day old males were not significant ($P=>0.1$).

Responses of ageing males to 18 day old females: 10 and 14 day old male responses showed no significant difference ($P=>0.1$) but 10 and 18 differed significantly ($P=<0.005$). Responses between 14 and 18 day old males also differed significantly ($P=<0.01$). Other groups were not tested because of lack of attraction in one or both the groups.

Responses of ageing males to 22 day old females: Differences in the response of 10 and 14 ($P=>0.05$) and 10 and 18 ($P=>0.1$) were not significant, but between 10 and 22 was significant ($P=<0.005$). Other group differences were not calculated due to lack of attraction.

Responses of 10 day old males to ageing females: Differences in the male response to 10 and 14 ($P=>0.1$) and 10 and 18 ($P=>0.1$) were not significant but between 10 and 22 day old females was significant ($P=<0.025$). Responses to 14 and 18 day old females was insignificant ($P=>0.05$), but between 14 and 22, and 18 and 22 day old females were significant ($P=<0.025$).

Responses of 14 day old males to ageing females: Attraction of males to 10 and 14 day old females was not significant ($P=>0.1$), but was significant between 10 and 18 ($P=<0.01$) and 10 and 22 ($P=<0.001$) day old females. Between 14 and 18 day old females there was no significant difference ($P=>0.1$) but between 14 and 22 was significant ($P=<0.005$). Differences between 18 and 22 day old females was not significant ($P=>0.1$).

Responses of 18 day old males to ageing females: Differences in the responses of 18 day old males to ageing females was similar to that of the 14 day old males except that the difference in attraction to 14 and 18 day old females was significant ($P=<0.05$) and between 18 and 22 day old females was not significant ($P=>0.1$).

Responses of 22 day old males to ageing females: Male response to 10 and 14 ($P=>0.1$) was not significant. Other groups were not calculated because of lack of attraction in one or both the groups.

FIG. 19

The effect of male and female age on the attraction of non-virgin males to non-virgin females.

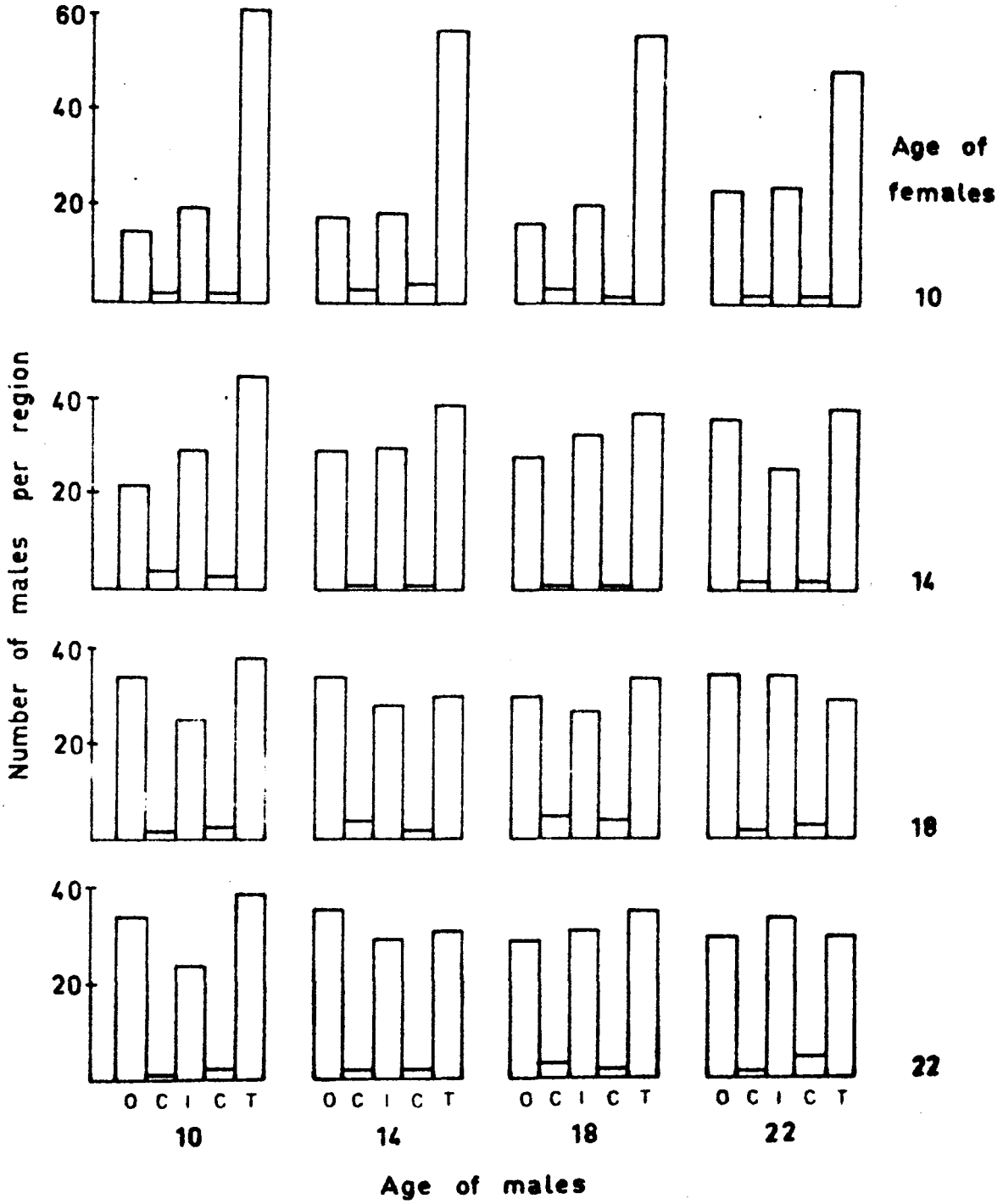

60
40
20
Age of females
10
Number of males per region
40
20
14
40
20
18
40
20
22
O C I C T
10
O C I C T
14
O C I C T
18
O C I C T
22
Age of males

FIG. 19

Attraction between non-virgin males and non-virgin females: (Fig. 19)

Only freshly copulated females (10 day old) were attractive to all age groups of males ($P=<0.025$). 14 day old females were not attractive to 18 and 22 day old males ($P=>0.1$) but 10 and 14 day old males were attracted ($P=<0.01$ and $P=<0.025$ respectively). 18 and 22 day old females did not attract males ($P=>0.1$).

DISCUSSION

Sex attraction in Chiloplacus symmetricus decreased with age and virgin females became unattractive towards the end of their life span. In his observations on Panagrellus redivivus, Duggal (1978a) also had observed that virgin females became unattractive as they aged but were attracted to males all through their life span. Hence, as females age, they apparently lose their potential of secreting attractants. Whether this was due to some physiological inhibition within the female or not, it nevertheless has immense evolutionary significance, as, ageing females when copulated with young males not only produced fewer eggs in C. lambdiensis but also showed a decreased reproductive period in T. aceti (Kisiel & Zuckerman, 1974). Teleologically speaking, we can say that the selection pressures may be responsible for causing a decline in the

attractiveness of females. Differences in attraction of ageing males to young females and vice versa, most probably result from a decrease in the activity of old males or perhaps from an increase in the threshold response.

Since freshly copulated females were responsive to all age groups of males, virgin and non-virgin, and 14 day old non-virgin females were also attractive to young virgin and non-virgin males, inhibition of sex attraction may not be solely due to copulation but may be its subsequent manifestations resulting most probably from changes in the female reproductive system which is most likely to be the source of attractants (Cheng & Samoiloff, 1972).

SEX ATTRACTION IN *CURZNEMA LAMBDIENSIS*

The effects of age and reproductive state of *Chiloplacus symmetricus* on sex attraction have been discussed earlier and Duggal (1978a) has observed that adult male *Panagrellus redivivus* showed no significant attraction towards copulated or gravid females although virgin females were attracted to males throughout their life span. In the following chapter, the sex attraction behaviour of *Curznema lambdiensis* is discussed.

MATERIALS AND METHODS

The nematodes were cultured on peptone-agar supplemented with wheat flour. Sex attraction was studied in modified mickey mouse traps.

Attraction of males to males and females to females: Males and females were separated during the fourth moulting stage and were reared in separate Petri dishes. Fifty males to be tested for attractants, were kept in a plastic straw pipe containing agar and placed in the centre of the test chamber for 6 hr to allow gradients to develop. 100 males were then released in the inoculation chamber and their distribution was recorded after 3 hr. A similar experiment was done to study the attraction of females to females.

Sex attraction was also tested between young and old virgin males and virgin females, virgin males and non-virgin females and between non-virgin males and virgin females.

Effect of the number of worms on sex attraction: Attraction of virgin males and virgin females was tested by using an increasing number of worms in the following order; 10, 50, 100, 150 and 200. The final distribution of the worms was recorded after 3 hr.

Effect of male and female ratio on sex attraction: Attraction of virgin males and females to varying ratios of 1:50, 10:50, 20:50, 30:50, 40:50 and 50:50 was studied in mickey mouse chambers. The two sexes were kept in a straw pipe in the test chamber and allowed to mix.

The above experiments were replicated five times except the one involving attraction to varying number of worms. This was replicated only three times.

RESULTS

Attraction of males to males and females to females: (Fig.20A,B) Virgin males did not show any significant attraction towards virgin males ($P=\rangle 0.1$) and virgin females also did not respond to virgin females ($P=\rangle 0.1$).

FIG. 20

A - The response of males to males ($P = > 0.1$).

B - The response of females to females ($P = > 0.1$).

C - The response of non-virgin males to virgin females ($P = < 0.001$).

D - The response of virgin females to non-virgin males ($P = < 0.05$).

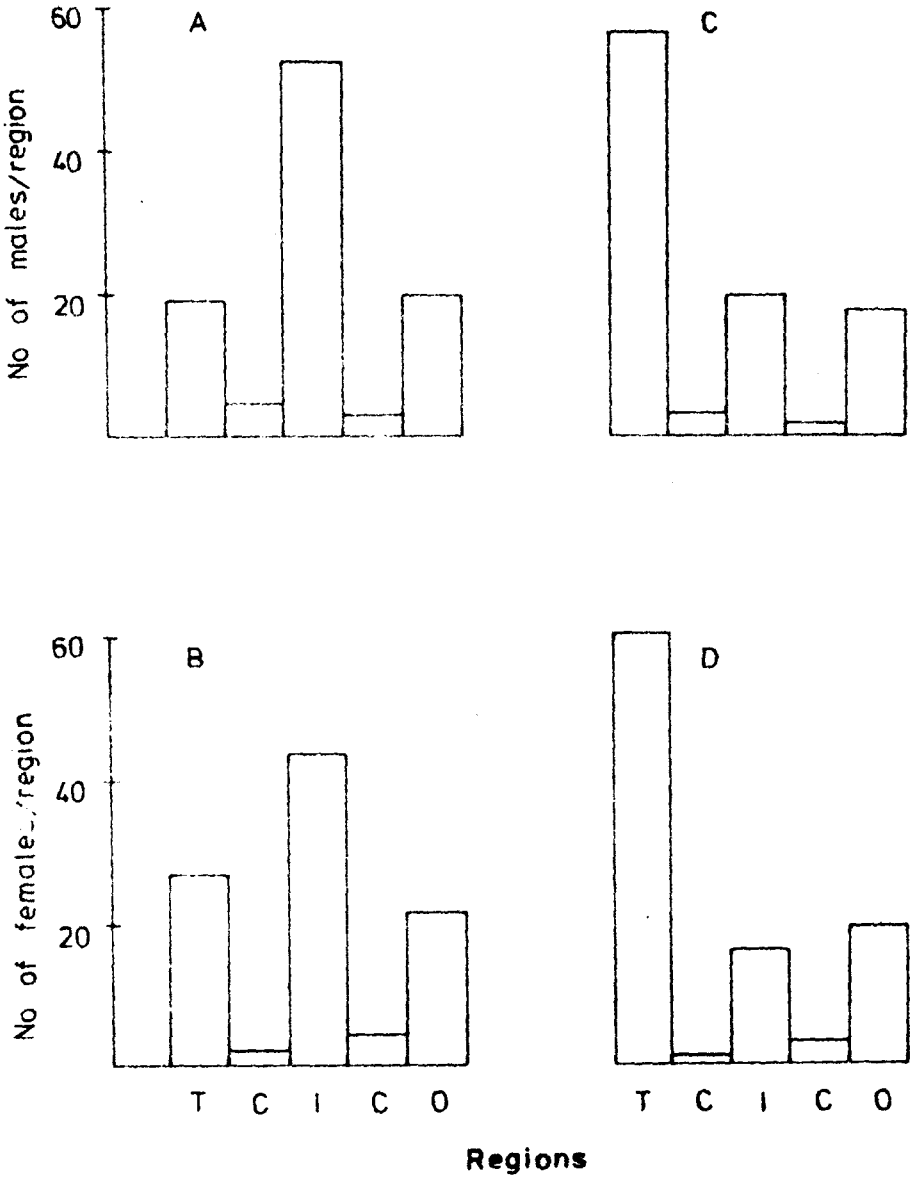

60
40
20
No of males/region
A
C
No of female/region
B
D
T C I C O
T C I C O
Regions

FIG. 20

Attraction between virgin males and virgin females: (Fig. 21A-D)

Young (1-2 day old) virgin males were attracted to young virgin females of the same age ($P = < 0.001$) but did not respond to 6-7 day old virgin females ($P = > 0.05$). Young virgin females were also attracted to young virgin males ($P = < 0.001$) and also showed a positive response towards 6-7 day old virgin males ($P = < 0.01$).

Attraction between virgin males and non-virgin females: (Fig. 21 E,F)

Virgin males were not attracted to 2-3 day old non-virgin females ($P = > 0.1$), but non-virgin females showed a positive response towards virgin males ($P = < 0.01$).

Attraction between non-virgin males and virgin females: (Fig. 20 C,D)

Non-virgin males showed a significant attraction towards young virgin females ($P = < 0.001$). Virgin females were, however, also attracted to 1-2 day old non-virgin males ($P = < 0.05$).

Effect of the number of worms on sex attraction: Attraction of males to varying number of females and vice-versa showed a similar pattern (Fig. 22). In both cases, attraction was positive but minimum at an attractant dosage of ten worms and

FIG. 21

A - The response of young virgin males to young virgin females ($P = < 0.001$).

B - The response of young virgin females to young virgin males ($P = < 0.001$).

C - The response of young virgin males to old virgin females ($P = > 0.05$).

D - The response of old virgin females to young virgin males ($P = < 0.01$).

E - The response of virgin males to 2-3 day old non-virgin females ($P = > 0.1$).

F - The response of 2-3 day old non-virgin females to virgin males ($P = < 0.01$).

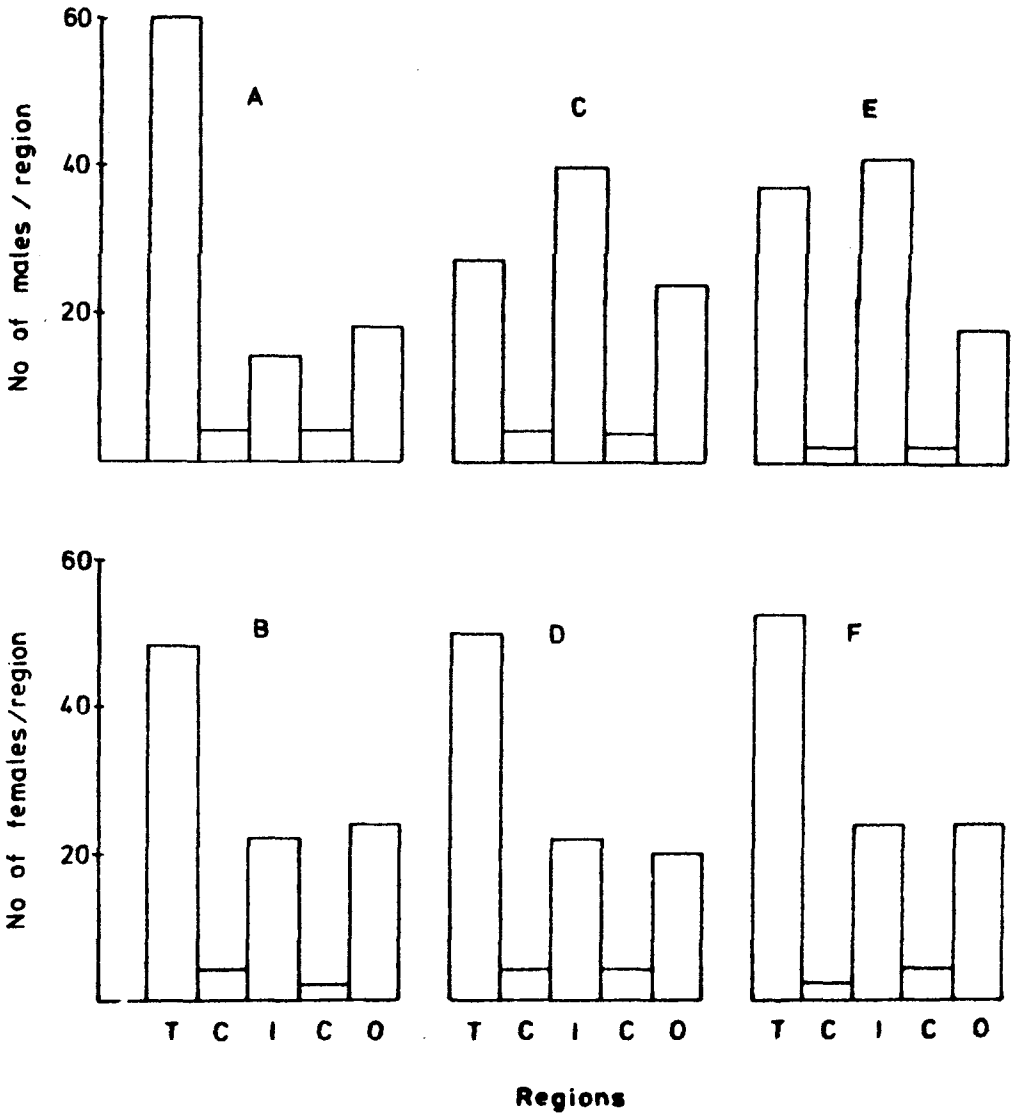

60
40
20
No of males / region
A
C
E
60
40
20
No of females/region
B
D
F
T C I C O
T C I C O
T C I C O
Regions

FIG. 21

FIG. 22

The effect of number of attractant worms on sex attraction.

FIG. 23

The effect of male and female ratio on sex attraction.

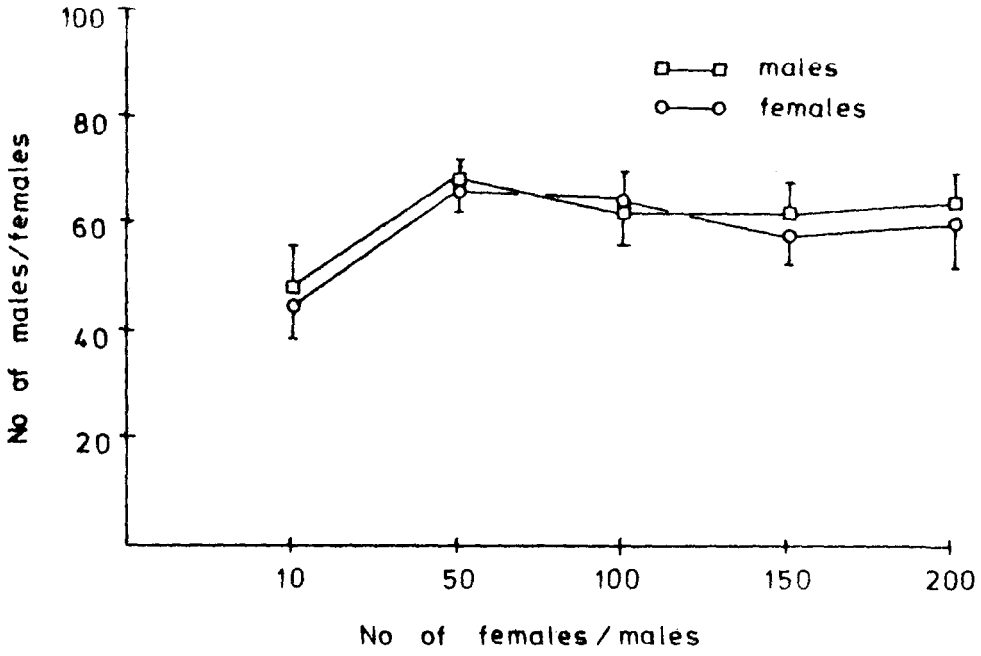

100
80
60
40
20
No of males/females
males
females
10
50
100
150
200
No of females / males

FIG. 22

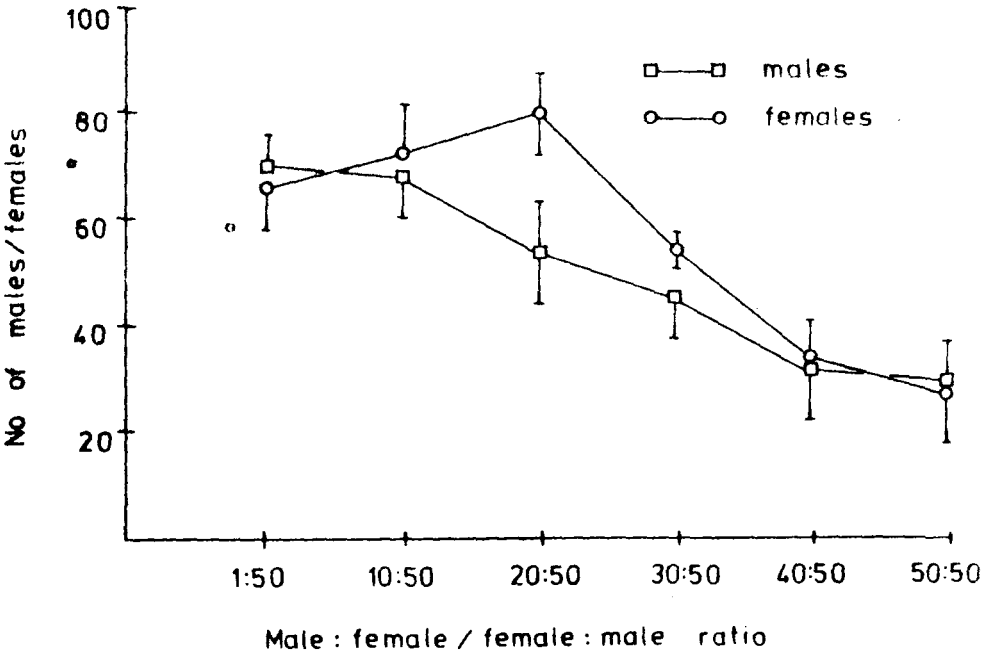

100
80
60
40
20
No of males/females
males
females
1:50
10:50
20:50
30:50
40:50
50:50
Male : female / female : male ratio

FIG. 23

maximum at 50 worms. The increase in response was significant ($P = <0.01$). However, further increase in the number of worms produced no corresponding significant ($P = >0.1$) increase in attraction.

Effect of male and female ratio on sex attraction: (Fig.23)

The female response at female: male ratio of 1:50, 10:50 and 20:50 was positive and showed a gradual though not significant increase ($P = >0.05$). Female: male ratios of more than 20:50 resulted in a sudden decrease in attraction and the female response was not significant at ratios of 40:50 and 50:50 ($P = >0.1$). Differences in the response of females to males alone (Fig. 22) and to 20:50 female: male ratio was significant ($P = <0.025$).

In contrast, males did not show an increase in attraction to any of the male: female ratios (Fig. 23). There was a gradual decrease in attraction and at 30:50 male: female ratio males were not attracted ($P = >0.1$).

DISCUSSION

Like *Panagrolaimus rigidus* (Greet, 1964), *Panagrellus silusiae* (Cheng & Samoiloff, 1971) and *P. redivivus* (Duggal, 1978a), both sexes of *Curznema lambdiensis* produced attractants

which resulted in a mutual response towards each other. Virgin females lost their attractiveness as they grew old while non-virgin females apprarently became unattractive after copulation. These observations are similar to those of Duggal (1978a) for P. redivivus except that he did not test females to virgin and non-virgin males separately. As he took males directly from the culture, presumably, they were non-virgin and since virgin females were attracted to them throughout their life span, it may be concluded that non-virgin males produced attractants throughout their life as in the present study with C. lambdiensis. Duggal (1978a) concluded that P. redivivus females were attractive or attracted to males only when they had no sperm and large number of oocytes in their oviduct. As female response is dependant on the presence of male attractants and as males produce attractants throughout their life span it becomes apparent that a 'no response' by the females is brought about by a blockage in the chemosensory receptors. For such a feed back system to operate, the reproductive system should be connected to the central nervous system. However, Yuen (1971) failed to observe nervous tissues in the reproductive system of Aphelenchoides blastophthorus. After the above experiments with C. lambdiensis it is suggested that perhaps copulation initiated a change in the reproductive tract of the female that ultimately inhibited production of sex

attractants. This suggestion is, however, based on the study of Cheng & Samoiloff (1972) where they showed that inhibition of gonad development also inhibited sex attraction in P. silusiae and hence the gonads were the source of the attractants. As 6-7 day old virgin females also became unattractive, there is a possibility that degenerative changes in the gonad might also inhibit production of sex attractants.

In C. lambdiensis like in C. symmetricus and P. silusiae (Balakanich & Samoiloff, 1974) males and females show a maximum, response at a particular optimum concentration of attractant (Fig. 22). However, the synergistic effect of female: male ratio of 20:50 on the attraction of females is here reported for the first time in a free-living nematode and it appears rather perplexing because as shown above, like sexes do not attract each other. It is quite possible that this increased attraction occurred either due to a mixing of the male and female pheromones or it resulted due to some other secretions that might have been produced by one or both the sexes upon physical contact. The inhibitory effect of male on male is already known in another species, N. brasiliensis (Bone & Shorey, 1977) but whether it is due to any secretion has not yet been established. Further, the synergistic attraction as evidenced in the present species is in direct contrast to that of N. brasiliensis. In the latter

species, when males were kept with females, (either in contact or separately) there was a gradual decrease in attraction, but it increased when the number of males was either equal to or more than the females. While further work may be necessary to explain this interesting phenomenon, but it nevertheless appears to be of immense value in the survival of the two species. N. brasiliensis which is an animal-parasitic nematode avoids overcrowding by this phenomenon while C. lambdiensis which is a free-living nematode, ensuring successful propagation of the species.

THE COPULATORY BEHAVIOUR OF *CURZNEMA LAMBDIENSIS*

Not much work has been done on the copulatory behaviour of plant and soil nematodes, and in that too the authors have only described the mechanism of copulation (Greet, 1964; Jones, 1966; Chin & Taylor, 1969). Recently, Duggal (1978b) gave a rather detailed account of the copulatory behaviour of the free-living nematode, *Panagrellus redivivus* and showed that the rate of copulation was related to the rate of sperm transferred. In *Rhabditis pellio* the number of matings decreased with age (Sommers *et al.*, 1977), while in *Aphelenchus avenae* the intervals between copulation increased with age (Fisher, 1972). In the present work, the copulatory behaviour of young and ageing *Curznema lambdiensis* has been studied in detail.

MATERIALS AND METHODS

The nematodes were cultured in peptone-agar supplemented with wheat flour.

Copulatory behaviour: The copulatory behaviour was studied in micro-chambers. Newly moulted adult males were placed singly with 4-5 newly moulted adult virgin females and were observed under the microscope. Besides this, observations on mating were recorded in cultures.

Rate of copulation and sperm transfer: To determine the rate of copulation and the number of sperm transferred, freshly moulted adult males were kept separately with ten freshly moulted adult females in a Petri dish containing 1% water agar. The females were removed after 12 hr, examined and a fresh batch of virgin females was added again to the Petri dish. The males were observed till they died.

Effect of isolation of male on copulation and sperm transfer: Ageing effects of males on copulation and sperm transfer were studied by placing 1, 2, 3 and 4 day old virgin males with 10 one day old virgin females in a Petri dish containing 1% water agar. The females were examined every 12 hr, after which a fresh batch of virgin females were added to the Petri dish. Males were transferred to fresh media every 24 hr and observed till they died.

Sperm transferred on first copulation: To study the number of sperm transferred on the first copulation, males were placed singly with 8-10 virgin females in a Petri dish and observed continuously under a binocular microscope. Tests were carried out with males isolated for 0, 1, 2 and 3 days. The time elapsed till the first copulation was also recorded.

There were five replicates for each set of experiments.

RESULTS

Copulatory behaviour

Location of the vulva and attachment to the female: When an active male came in contact with a female, it at once pressed its tail against the female body and moved over her backwards. If the male touched the female with its anterior end, there were quick back and forth movements giving the impression of searching behaviour. This, however, was not of consistent occurrence and was exhibited only by those males which were intending to copulate. In cultures, the males could be divided into two distinct groups: i) those that appeared to be apparently unaware of the presence of females in the neighbourhood and continued to feed without attempting to copulate, and ii) those which actively attempted copulation. Once the male tail had pressed against the female body, the spicules probed the female regularly, both the spicules working together.

For a successful mating it was essential that the male and female body should be properly orientated with respect to each other, i.e., they should point (with anterior ends) in opposite directions (Fig. 24) and their ventral surfaces should be mutually opposed. No mating was observed with both the sexes pointing in the same direction. The males apparently showed no preference for virgin females and copulated as readily with gravid females, although the number of sperm transferred differed considerably.

FIG. 24

Copulating postures in *Curznema lambdiensis* males.

Penetration: Once the spicules had located the vulval opening, the female was held firmly. The spicules made repeated thrusts into the vagina and penetrated deep into it. The males stopped feeding at this stage.

Insemination: The males produced anteriorly directed waves of the body soon after penetration which resulted in the shortening of the body and an increase in the internal body pressure. Simultaneously, the body swept across in wide arcs with the anterior region oscillating vigourously (Fig. 24). Just prior to the release of sperm, all activities ceased and the male lay motionless for a few seconds with its spicules inserted into the vagina. Soon afterwards, there was a simultaneous retraction of the spicules and shortening of the body which resulted in the flow of sperm into the uterus. On the average, 20-33 sperm were transferred per copulation (Fig. 26) except in the case of six day old males where fewer sperm were transferred.

The intensity of the sweeping movements and the body contractions were dependant on the age and reproductive state of the males, being greater and more intense in normally copulating 3-4 day old males than in 1-2 day old males.

Behaviour of the males when disturbed during copulation: The males displayed consistently distinct behavioural characteristics

depending at what stage of copulation they were disturbed. In the early stages, before the penetration of the spicules, if the female moved out of its grip, the males showed sharp reversals alternating with forward movement perhaps indicating searching behaviour. If it could not make contact again, it started feeding. If separated after spicule penetration and the subsequent shortening of the body, they became motionless with the spicules protuded. Occasionally, a few sperm were discharged out of the body passively. The males lay motion less for about 50 min and afterwards resumed activity with slight movements first in the anterior end and then gradually spreading over to the entire body. The spicules were, however, retracted only when the male had moved a short distance away.

Behaviour of the females during copulation: All through the process of copulation, the females continued feeding with indifference. Occasionally, when the male probed its anterior end, there was a sharp withdrawal response. After insemination, when the male had moved apart, vulval twitchings were observed both in the virgin as well as gravid females.

Rate of copulation and sperm transfer: The mean rate of copulation and the number of sperm transferred were closely related over the entire six day period (Fig. 25). During the

first five days, the mean number of copulations per day varied from 3-7.2 and the mean number of sperm transferred from 61-176. The mean number of sperm transferred per copulation per day ranged from 20-33 (Fig. 26). Difference between the maximum number of sperm transferred per day (on day two) and the minimum per day (on day five) was statistically significant ($P = <0.025$). Only two of the five males copulated on day six and hence it is considered to be of abnormal occurrence and no inferences are drawn from it. Comparisons between the mean number of copulations and the mean number of sperm transferred per copulation per day showed an inverse relationship for the first three days and then a direct relationship (Fig. 26). Hence, more sperm were transferred when there were few copulations than when there were more.

Normally copulating males which were allowed to copulate freely after the final moult copulated 15-32 times and transferred a total of 517-754 sperm in their life span.

Effect of isolation of male on copulation and sperm transfer: As the isolation of the males increased beyond two days, both the total number of copulations and the number of sperm transferred decreased significantly (Fig. 27; $P = <0.001$). Males isolated for four days failed to inseminate females and only in one case copulation was observed once. Males isolated from females became

FIG. 25

Rate of copulation and the number of sperm transferred in the life span of *Curznema lambdiensis* males.

FIG. 26

The effect of age of male on the number of sperm transferred per copulation per day and the number of copulations per day.

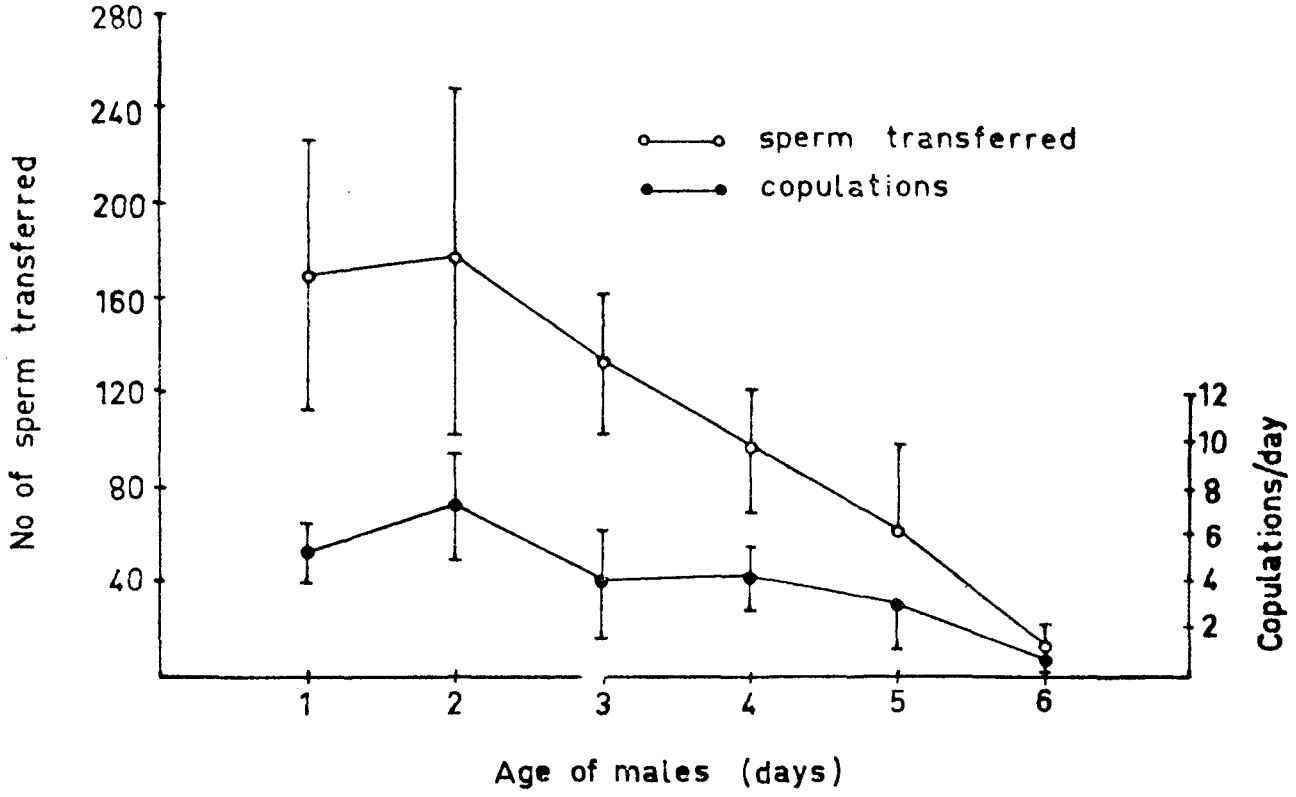

No of sperm transferred
280
240
200
160
120
80
40
sperm transferred
copulations
12
10
8
6
4
2
Copulations/day
1
2
3
4
5
6
Age of males (days)

FIG. 25

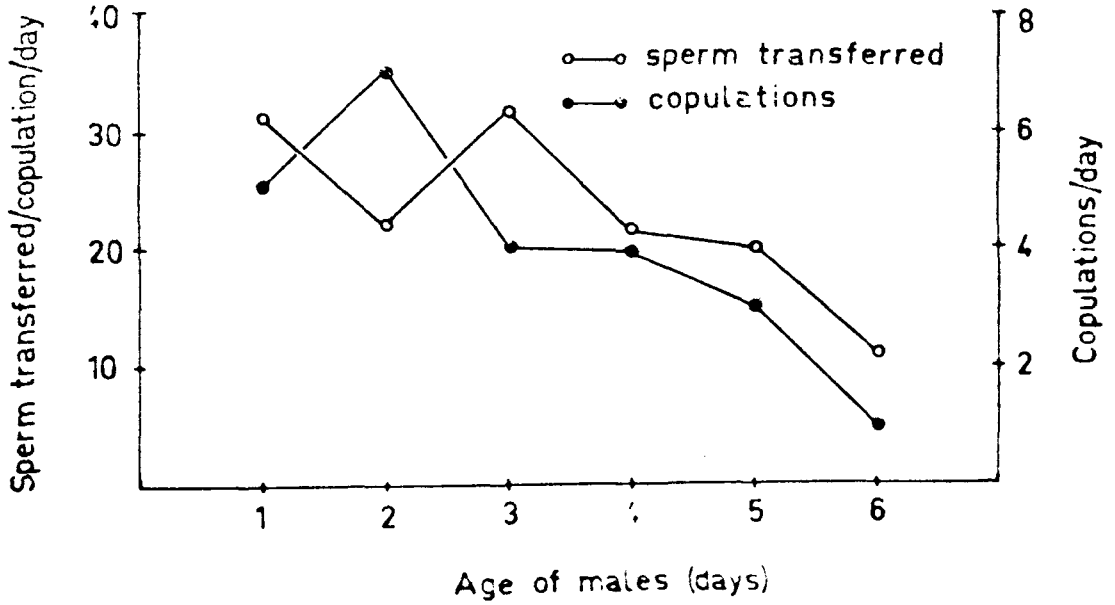

Sperm transferred/copulation/day
40
30
20
10
sperm transferred
copulations
8
6
4
2
Copulations/day
1
2
3
5
6
Age of males (days)

FIG. 26

sensitive and usually displayed a sharp withdrawal response when they made their first contact with females. Further, in males isolated for more than two days, the sperm accumulated in the seminal vesicle began to show degenerative changes.

Number of sperm transferred on the first copulation: The mean number of sperm transferred on the first copulation by isolated virgin males varied from 34-72. The maximum number of sperm were transferred by males isolated for one day. Virgin males isolated for two or three days showed a significant decline in the mean number of sperm transferred on the first copulation ($P = < 0.05$; $P = < 0.005$ respectively) from one day isolated males. The mean number of sperm transferred on the first copulation by zero and one day isolated males was more ($P = < 0.025$) than the mean number of sperm transferred per copulation per day (Fig. 28). However, the mean number of sperm transferred by two and three day isolated males did not differ significantly. ($P = > 0.1$).

Isolation of virgin males resulted in a gradual increase in the time required for the first copulation (Fig. 29). Differences between one and zero and three day isolated males was significant ($P = < 0.001$).

FIG. 27

The effect of isolation of males on the total number of sperm transferred and the number of copulations.

FIG. 28

The effect of isolation of males on the number of sperm transferred on the first copulation and number of sperm transferred per copulation per day.

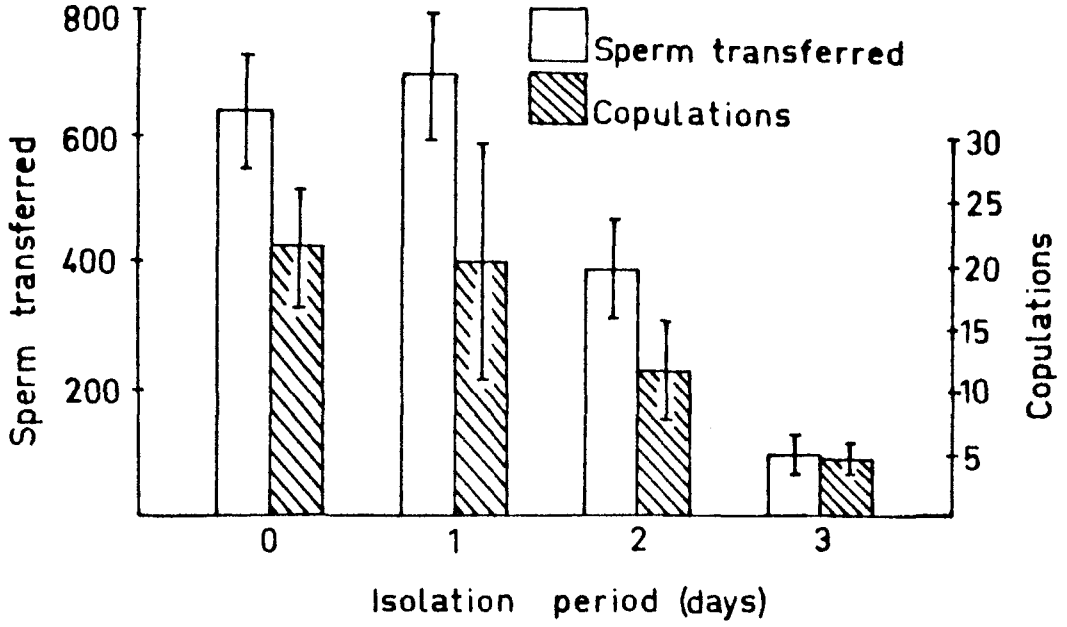

800
600
400
200
Sperm transferred
Sperm transferred
Copulations
30
25
20
15
10
5
Copulations
0
1
2
3
Isolation period (days)

FIG. 27

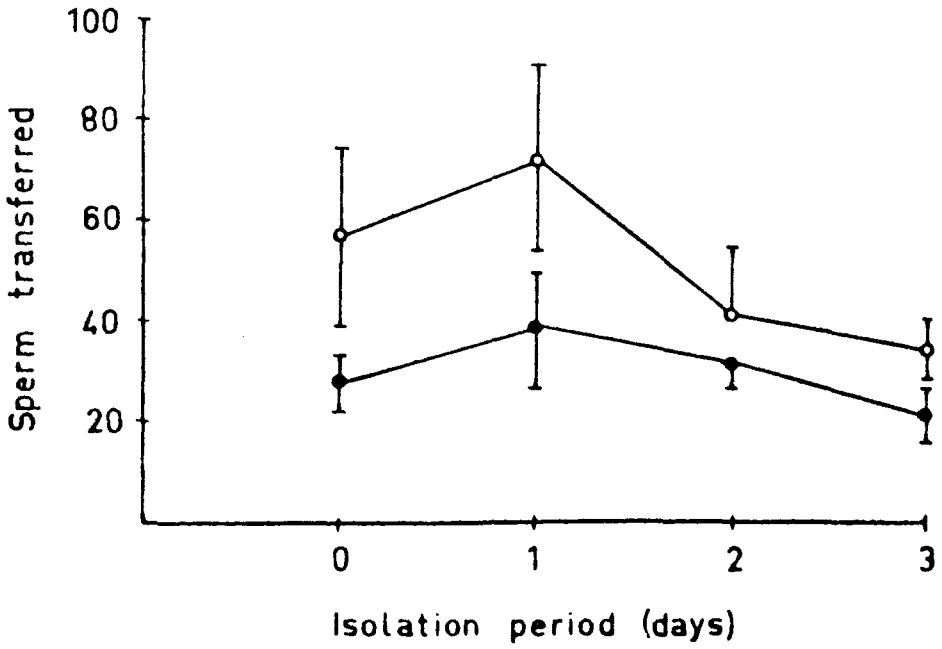

100
80
60
40
20
Sperm transferred
0
1
2
3
Isolation period (days)

FIG. 28

FIG. 29

The effect of isolation of males on the time required for the first copulation.

Fitted regression line y= 21.8 + 17.6x

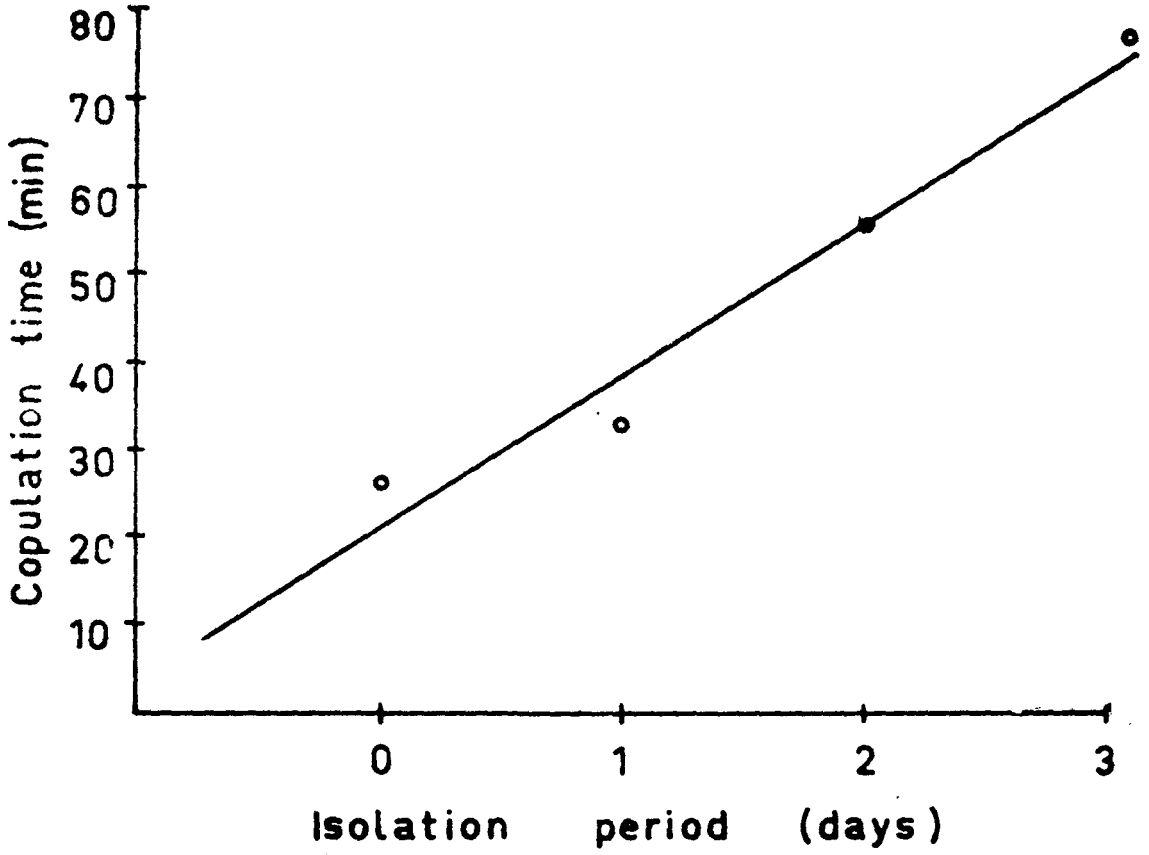

80
70
60
50
40
30
20
10
Copulation time (min)
0
1
2
3
Isolation period (days)

FIG. 29

DISCUSSION

Studies on the copulatory mechanisms of nematodes have shown that the spicules were inserted into the vagina during copulation and aided in the transfer of sperm from the male to the female genital tracts (Greet, 1964; Jones, 1966; Chin & Taylor, 1969; Fisher, 1972; Duggal, 1978b). Further, ultrastructural studies of the male copulatory apparatus have revealed that either the vela membranes or the spicules themselves form a tubular structure through which sperm pass during insemination (Clark et al., 1973; Hogger & Bird, 1974; Wen & Chen, 1976; Clark et al., 1977; Duggal, 1978b). The formation of a tube by the flanged spicules of Ancylostoma was considered by Looss (1905) to help in the flow of sperm. But in species where the spicules were not flanged and a tube was not formed, it was suggested by Mueller (1930) that the spicules were withdrawn during the movement of sperm. The mechanism of sperm transfer in C. lambdiensis seems to add weight to the suggestion of Mueller (1930). However, a more detailed study of the spicules and associated structures is required to understand the actual mechanism of insemination. In C. lambdiensis the males have a very strongly developed bursa and well developed cloacal lips which maintain the continuity of the male and female reproductive tracts even when the spicules are withdrawn.

The frequency of copulation and the number of sperm transferred by normally copulating males began decreasing after two days and similar results were obtained when males were isolated for varying periods of time. Hence, both these activities may be dependant on the physiological state of the males. Duggal (1978b) also observed a significant decline in the frequency of copulation of long isolated males. The larger number of sperm transferred on the first copulation by two day old males could be due to the fact that these males had by that time accumulated a huge number of sperm in their reproductive tracts resulting in a larger number of sperm being released during copulation. A decrease in the sperm transferred by three and four day old males may in all probability be attributed to ageing effects. Both degeneration of sperm and weakening of the muscles of the vas deferens being responsible for the lower number of sperm transferred.

The inverse relationship between the mean number of sperm transferred per copulation per day and the mean number of copulations per day suggests that the more rapidly copulating males transferred fewer sperm than the slow copulating males.

The copulatory behaviour of C. lambdiensis is unique amongst the nematodes studied so far, but such studies have been few and a more detailed observations on behaviour and ultrastructure of the copulatory apparatus are necessary for a better understanding of this phenomenon.

AGEING AND REPRODUCTION IN CURZNEMA LAMBDIENSIS

Very little is known about the effects of ageing on the reproductive potential of nematodes. Fisher (1969) observed that under adverse conditions of temperature and food, the reproductive period of a parthenogenetic population of Aphelenchus avenae was prolonged, but the total number of eggs produced did not differ from that produced under normal conditions. In 1972 he observed that delayed mating in an amphimictic population of A. avenae reduced its egg production. Studies on ageing of Turbatrix aceti by Kisiel & Zuckerman (1974) have revealed that if mating was progressively delayed, the time prior to the first reproduction increased but the duration of the reproductive period was reduced. Duggal (1978c) showed that in ten day old virgin females of Panagrellus redivivus, the number of eggs produced within 24 hr was significantly reduced from that of the younger females.

MATERIALS AND METHODS

Changes in the reproductive system: Ageing changes in virgin males and females were studied by placing batches of fourth stage juveniles in separate Petri dishes and observing

them after every 24 hr until death. Observations on copulating worms were made by placing five male and five female juvenile stages in a Petri dish and examining every 24 hr. Copulating worms were transferred to fresh media every day and virgins every second day. 20 individuals of each sex were examined in the first case and 20 in the second.

Longivity and mortality rate: To determine the longivity and mortality rate of virgin worms, 50 freshly moulted males and 50 females were placed in separate Petri dishes and observed daily. Longivity of copulating worms was determined by placing 10 freshly moulted males with 10 freshly moulted females. The nematodes were transferred to fresh media every day to avoid overlapping of generations. There were five replicates.

Effect of female and male age on egg production: Virgin females 1, 2, 3, 4 and 5 days old were placed singly in five separate Petri dishes and to each were added five newly moulted males. The Petri dishes were examined every 24 hr and the number of eggs laid and juveniles hatched were counted. The males were replaced by a fresh batch of newly moulted ones every day, when the females were changed to fresh media. Each set was replicated

five times. In a similar manner, the effect of ageing males on reproduction was also studied. Freshly moulted females were placed with various age groups of males and the number of eggs produced was counted after every 24 hr. There were five replicates of each set. In all the cases the nematodes were studied till they died.

Effect of ageing on egg production on first copulation: Females of varying ages were placed singly with 5-6 freshly moulted adult males and observed continuously. After the first copulation the females were removed and placed in separate Petri dishes and the total number of eggs produced was recorded every 24 hr. In another set of experiments ageing males were placed singly with 5-6 freshly moulted adult females and were observed as above. After the first copulation the females were removed and placed in separate Petri dishes and the number of eggs produced was recorded. There were five replicates of each set.

RESULTS

The virgin female reproductive system: *Curznema lambdiensis* females are mono-opisthodelphic. A reflexed ovary leads into an oviduct which in turn joins with the spermatheca. From the distal part of the spermatheca arises the muscular uterus

which opens to the exterior via a short vagina and a transverse vulva. In one abnormal case, the ovary was intertwined with the oviduct and uterus (Fig. 30A).

In virgin females, mature oocytes detaching from the ovary quickly passed through the oviduct and after a brief stop in the spermatheca, entered the uterus coming to lie at the uterus-vagina junction (Fig. 30B). The total number of oocytes produced by one day old virgin females varied between 11-19. These unfertilized oocytes failed to develop an egg shell and were delimited only by a thin vitelline membrane. As the females grew older, the unfertilized oocytes usually ruptured filling the uterus with a dense granular mass. In 7-8 day old virgin females the entire uterus was filled with disintergrated egg masses (Fig. 30C). Some of it passed out occasionally with vulval twitchings but no regular excretion was observed. In older females this granular substance diminished. It is, therefore, highly probable that most of it was absorbed by the uterine wall. The ovary was eventually reduced to a shrivelled mass and the developing oocytes became vacuolated.

The non-virgin female reproductive system: In copulating female on an average 142 (102-171) eggs were produced on the first day. Sperm were stored in the seminal receptacle (Fig. 30 D) but when in excess, they passed into the oviduct. Even otherwise, a few sperm were always present in the oviduct and hence, the first

FIG. 30

The effect of ageing on the female reproductive system: A - Abnormal arrangement of female gonad showing the ovary coiled around the oviduct and uterus (x 200); B - Unfertilized oocytes at the distal part of the uterus (x 420); C - Uterus of old virgin female filled with dense granular egg mass (x 200); D - Oocyte entering the spermatheca containing sperm (x 600); E - Arrangement of eggs in the uterus of a young female (x 200); F - Developing eggs packed in the uterus of an old female (x 200).

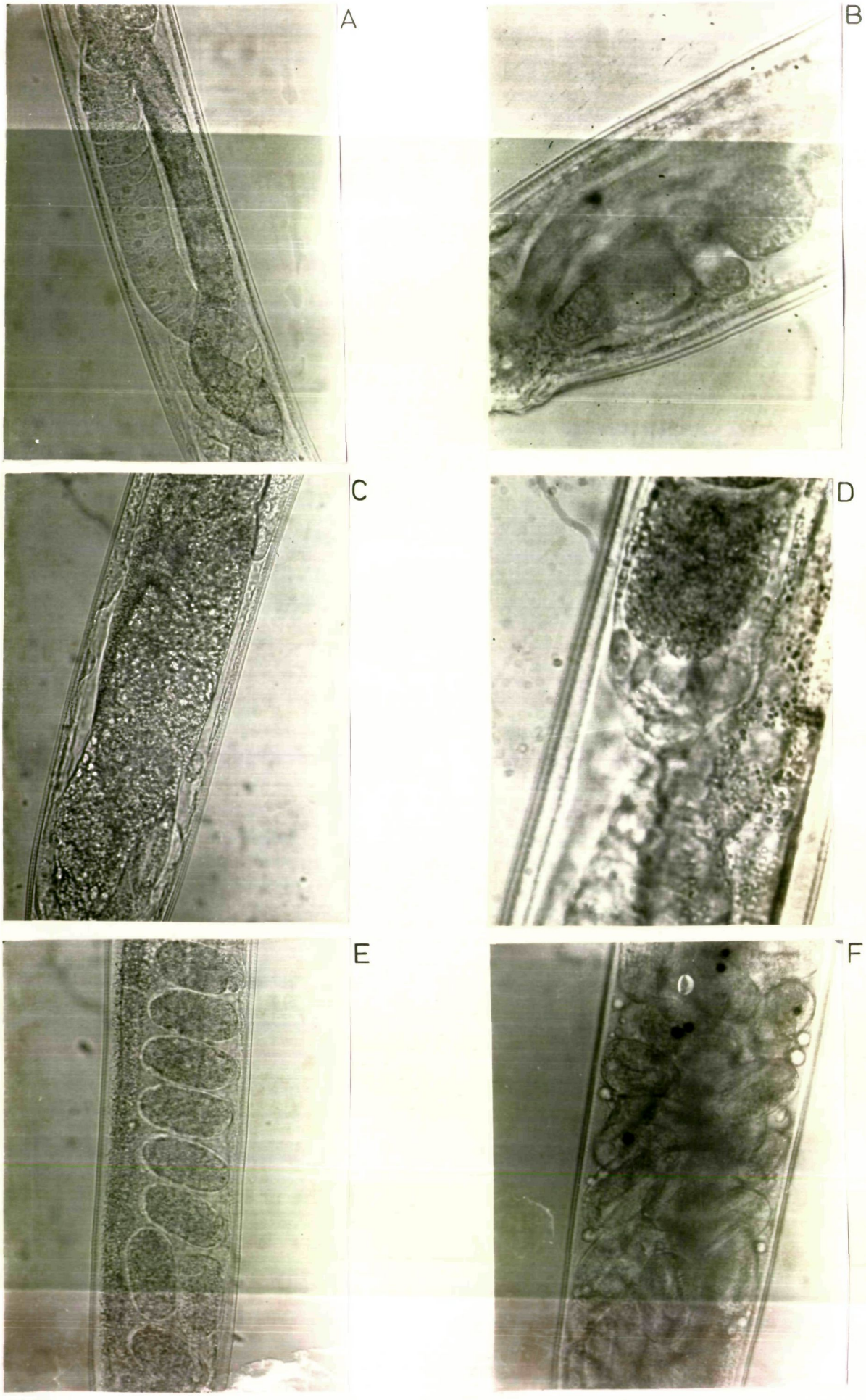


FIG. 30

contact of the egg with the sperm occurred here. The germinal cells proliferated rapidly and the reflexed ovary elongated posteriorly. Eggs were laid in batches and the uterus did not contain more than 21 eggs at a time (Fig. 30E). In ageing (5 days or more) females, however, the eggs were retained (Fig. 30F), ultimately leading to 'endotokia matricida' which was of common occurrence.

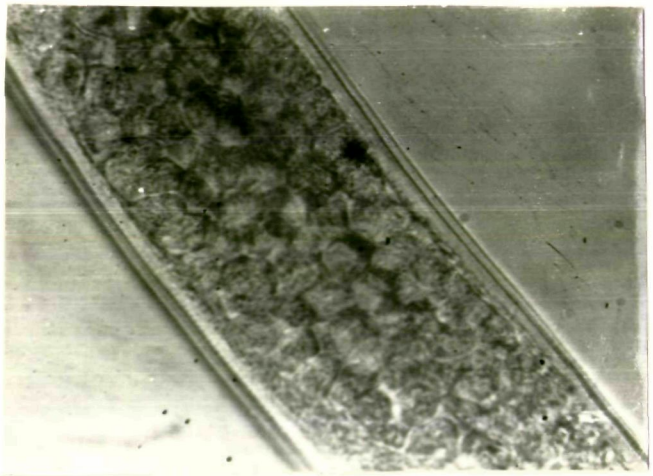
The virgin male reproductive system: Males are typically monorchic with a reflexed testis, a seminal vesicle and a vas deferens. The spicules are paired and straight and the tail is enveloped by a well developed bursa.

Spermatozoa begin maturing by the end of the final moult when they descend into the seminal vesicle. 12-15 hr after the final moult more than 100 sperm were present in the seminal vesicle. As the males aged the entire seminal vesicle became packed with sperm (Fig. 31A) so much so that the growth zone of the testis could hardly be differentiated. On the third day the sperm began to degenerate. They often became vacuolated or their entire cytoplasm condensed in the centre forming irregular bar-like structures (Fig. 31 B). Another characteristic abnormality was the mammilated appearance on the outer surface of the sperm (Fig. 31 C). Degenerate spermatozoa were probably reabsorbed

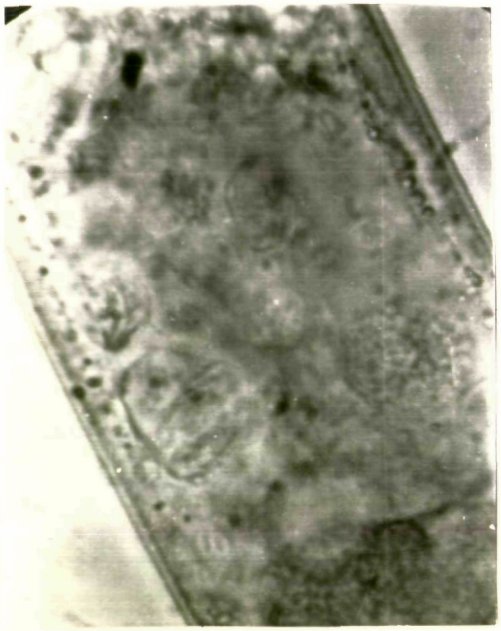
FIG. 31

The effect of ageing on the male reproductive system: A - Sperm packed in the seminal vesicle (x 420); B - An abnormal sperm showing condensed cytoplasm in an old male (x 600); C - An abnormal sperm with a mammilated surface in the seminal vesicle of an old male (x 600).

A



B



C

FIG. 31

into the walls of the seminal vesicle. The testis degenerated in the same manner as the ovary and the developing spermatocytes became vacuolated and finally the entire testis shrivelled up.

The non-virgin male reproductive system: In copulating males, as the spermatozoa were regularly discharged, accumulation did not occur. Degenerative changes in the reproductive system appeared on the third day. Sperm production decreased and the lumen of the vas deferens became wider probably due the weakening of the muscles. In its total life, a normal male transferred 517-754 sperm.

Longivity and mortality rate: The mean life span of virgin males and females was 10 days, while that of non-virgin worms was 6.5 days. In non-virgin worms, the mortality rate increased sharply after the fourth day (Fig. 32) and more than 90% of them died by the eighth day. The mortality rate of virgin individuals showed a gradual increase till the seventh day, but after that there was a rapid increase (Fig. 32).

Effect of male, female age on fecundity: When ageing virgin females were mated with young males, egg production showed a rapid decline (Fig. 33) and in five day old females no eggs were produced. Differences between successive days was significant till the fourth

FIG. 32

Mortality rate of Curznema lambdiensis non-virgin and virgin males and females.

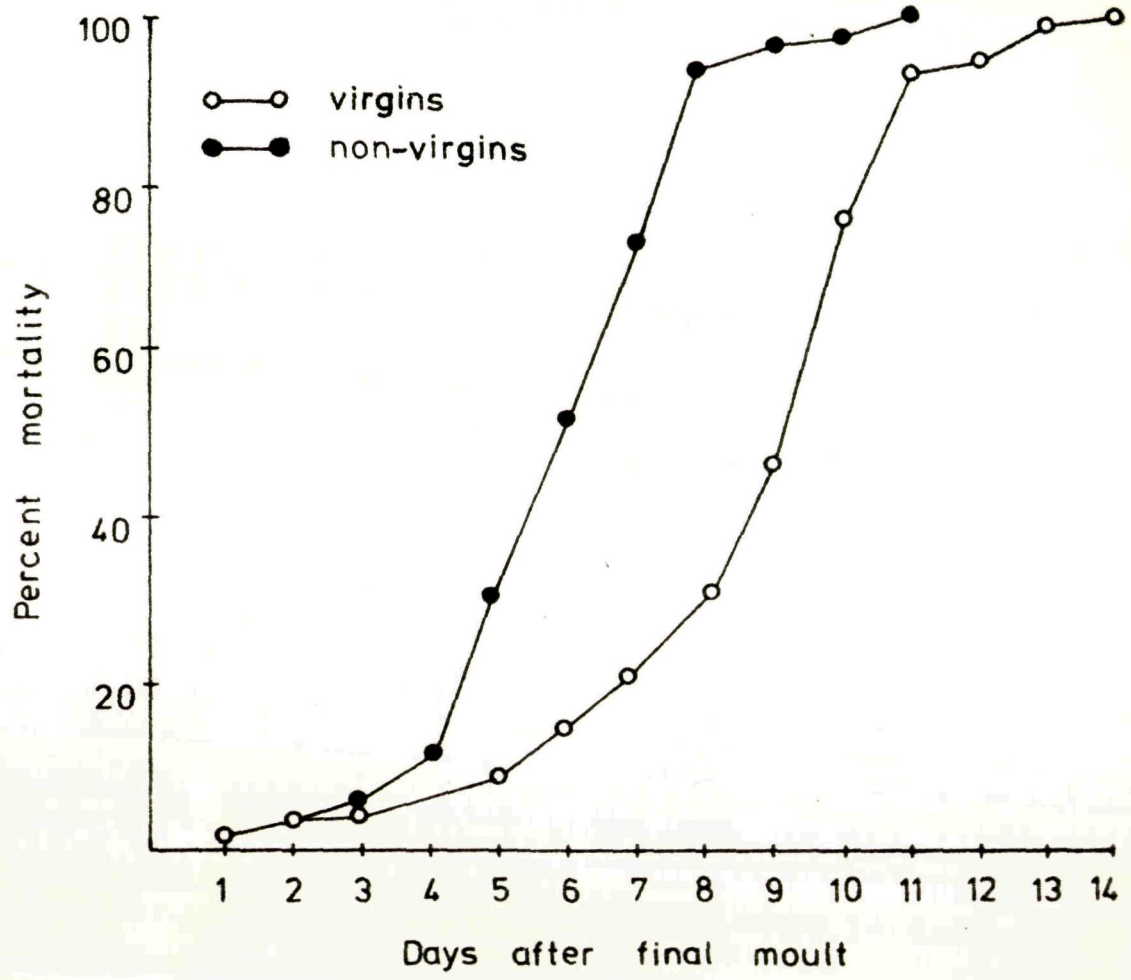

100
80
60
40
20
Percent mortality
virgins
non-virgins
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Days after final moult

FIG. 32

day ($P=<0.05$). Virgin females which had mated on the third or fourth day usually underwent 'endotokia matricida'. In younger females this occurred rarely.

When ageing males were mated with young females, the number of eggs produced on the first and second day was not significantly different ($P=>0.1$), but on the third and fourth day there was a sharp decline. Mean differences between the number of eggs produced on the second and third, and third and fourth day was significant ($P=<0.05$; and $P=<0.001$ respectively). No eggs were produced by five day old males and young females as the males failed to copulate.

Effect of ageing on egg production on first copulation:

The pattern of egg production when ageing virgin females were mated with young males showed a gradual decline (Fig. 34). There was a significant decrease on the third and fourth day ($P=<0.001$). When ageing males were copulated with young females, the number of eggs laid on the first and second day were almost equal (Fig. 34) but afterwards there was significant decline ($P=<0.001$). Differences in egg production on the third and fourth day between ageing females and ageing males were statistically significant ($P=<0.01$).

FIG. 33

The effect of male and female age on fecundity.

FIG. 34

The effect of male and female age on egg production after the first copulation.

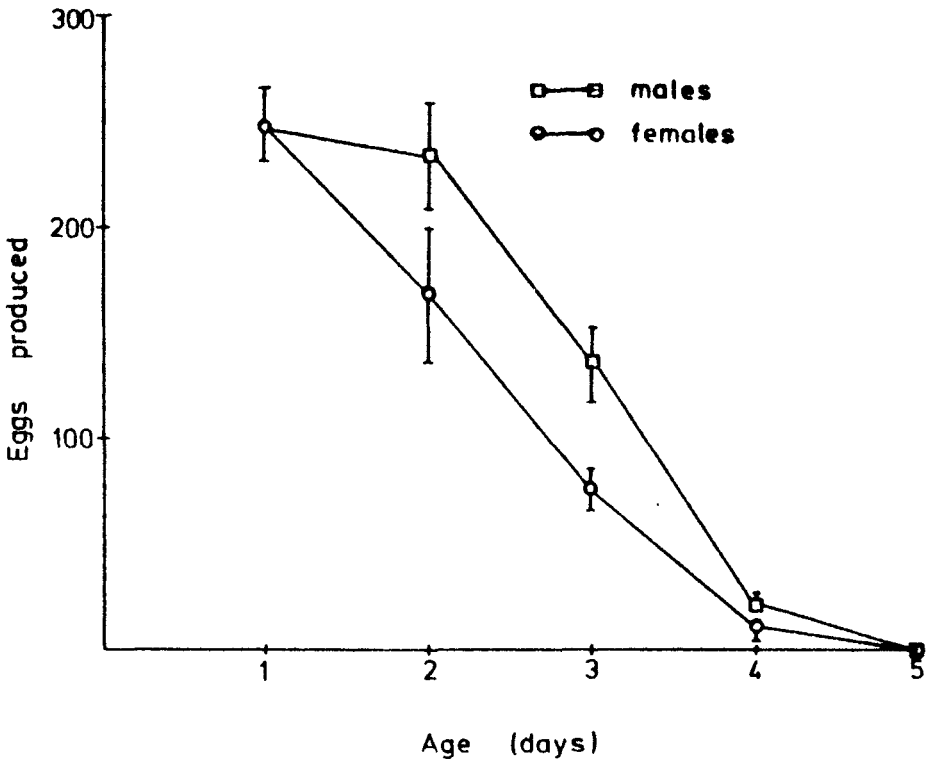

300
200
100
Eggs produced
males
females
1
2
3
4
5
Age (days)

FIG. 53

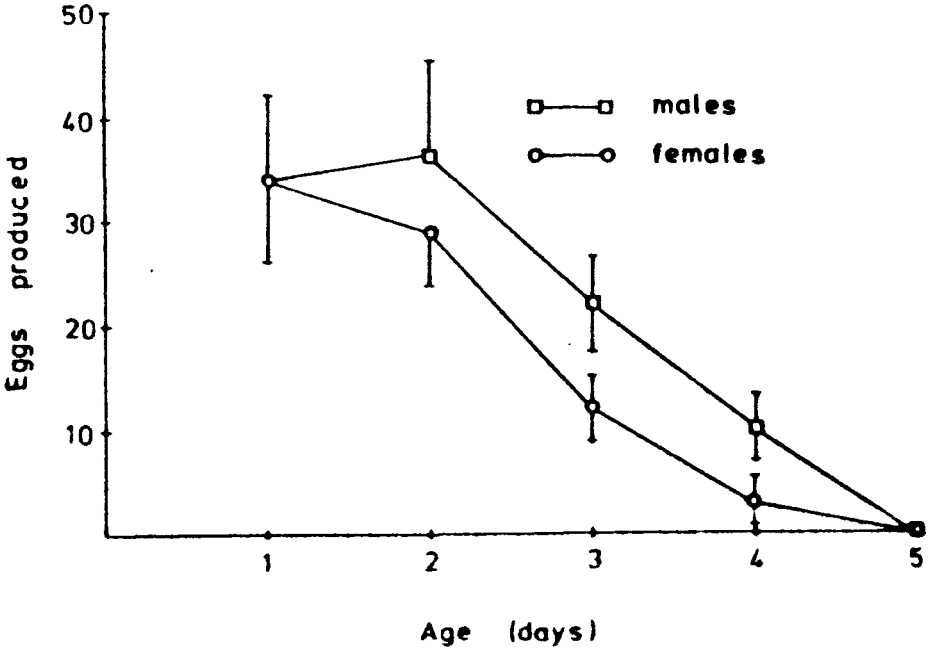

50
40
30
20
10
Eggs produced
males
females
1
2
3
4
5
Age (days)

FIG. 54

DISCUSSION

Duggal (1978c) in his observations on Panagrellus redivivus noted that in virgin females the oocytes were present in the uterus at the time of the final moult and they continued to increase subsequently. He, however, did not mention any structural abnormality of the unfertilized oocytes in the uterus. While development similar to P. redivivus also occurred in C. redivivus also occurred in C. lambdiensis, the oocytes which passed into the uterus failed to develop an egg shell and consisted of irregular egg masses enveloped by only a thin vitelline membrane. The development of the egg shell therefore, appeared to be conditional and was secreted by the egg itself after fertilization had taken place. Non-viable eggs were not laid but disintegrated within the uterus and were probably reabsorbed. Reabsorption of unfertilized oocytes has also been suggested by Duggal (1978c) in virgin females of P. redivivus, but in Ascaris lumbricoides, the uterus has cells which were found to be phagocytotic engulfing unused ageing spermatozoa (Romieu, 1911; Romies, 1913).

Decrease in egg production in ageing females probably resulted from obstruction of spermatozoa by the granular mass of disintegrated oocytes from reaching the spermatheca. Secondly, not all the sperm present in the spermatheca and/ or oviduct were able

to fertilize the oocytes as is evident from the fact that the mean number of sperm transferred on the first copulation by one day old virgin males was 56 but the mean number of eggs produced by females was much lesser (Fig. 34) and decreased with age of the female. As the number of eggs produced by young females when mated with ageing males (Fig. 33) and also the number of eggs produced after the first copulation under similar conditions of the sexes, on day three and four were significantly different from the number produced by ageing females when copulated with young males, it may be possible that the granular mass present in older females might also have a toxic effect on the sperm thus reducing its life span.

ANALYSIS OF THE COPULATORY SENSES OF CURZNEMA LAMBDIENSIS

In cultures of C. lambdiensis it was observed that the males either actively attempted to copulate or fed with indifference alongside nubile females. While the age and reproductive state of the worms may be an important factor influencing copulation (Duggal, 1978b) it may not be the only reason for this diverse behaviour. Not much work has been done on the copulatory senses of nematodes besides the demonstration of nerve tissues in the associated copulatory structure (Clark et al., 1973; Lee, 1973; Hogger & Bird, 1974; Clark et al., 1977). In the present work, an attempt has been made to analyse the copulatory senses of C. lambdiensis.

MATERIALS AND METHODS

Discriminatory ability of males: A 5.5 cm diam Petri dish was half-filled with 1% water agar and allowed to solidify. Pieces of glasswool 1-2 mm long and with thickness almost equal to the female body were scattered on the surface of the agar. Three groups of males viz., one day old virgins, three day old virgins and normally copulating males were tested for copulation. From each group a single male was released on the agar and allowed to make 50 contacts with the glasswool. During these contacts,

the number of times the males attempted copulation was recorded on a tele-counter. An attempted copulation was recorded when the male pressed its tail against the glasswool and moved over backwards simulating the copulatory proceedure. There were ten replicates for each set of males.

In the second experiment, dead females (freshly killed in warm water) were placed on the surface of agar and again the males were released and observed as before. Each set of males was replicated ten times.

In the third experiment, males were placed with live females and their behaviour was studied similarly. Males were only allowed to make contact but not allowed to copulate. There were ten replicates of each set of males.

Effect of sex attractant gradient on the copulatory behaviour: In this case, three sets of experiments similar to those done above were performed. However, before the experiment, an attractant gradient was allowed to develop on the agar. Other details were the same.

Copulatory pattern: Freshly moulted adult males were placed singly with 25 freshly moulted females in a Petri dish. The dishes were examined every hour to see if the females had copulated and those that had mated were removed and fresh ones added. The

number of sperm transferred and the timing of each copulation was noted. The copulatory pattern of three groups of males was observed: i) freshly moulted adult males; ii) males isolated for 48 hr; iii) males that were isolated for 14 hr and then kept with females for 10 hr. Three males were studied for each group.

RESULTS

When males made contact with dead or live females, they either showed an indifferent behaviour, being quite oblivious of the presence of the other nematode, or they actively attempted to copulate. Their reaction to small pieces of glass-wool was somewhat similar although very frequently they showed a withdrawal response before proceeding forward again.

Behaviour on plain sterile agar.

Two day old virgin males: Copulation was attempted significantly fewer times with glasswool than with dead ($P=<0.01$) or live ($P=<0.05$) females, but the number of attempts with dead and live females was not significantly different ($P=>0.1$).

Freshly moulted males: Freshly moulted virgin males showed a similar pattern of behaviour i.e., differences between attempts on glasswool and dead and live females were significant

($P=<0.01$) but it was not so between dead and live females ($P=>0.1$). The behaviour of freshly moulted males towards any of the test material was not significantly different from two day old virgin males ($P=>0.1$).

Normally copulating males: In these groups of males also, the differences between attempted copulations with glasswool was significantly less than with dead females ($P=<0.05$) or live females ($P=<0.01$) but there was no significant difference between dead and live females ($P=>0.1$). The differences in copulatory attempts on glasswool was not different between two day old virgin males and normally copulating males or between freshly moulted males and normally copulating male ($P=>0.1$), but with dead females the differences between both the above groups was significant ($P=<0.025$; $P=<0.05$ respectively). With live females, however, there were no significant differences ($P=>0.1$).

Behaviour on agar with attractant gradient

Two day old virgin males: Two day old virgin males showed a significant increase in attempted copulation with dead and live females than with glasswool ($P=<0.025$; $P=<0.05$ respectively). There was no significant difference between dead and live females ($P=>0.1$).

FIG. 35

A - Attempted copulations by males on plain sterile agar.

B - Attempted copulations by males on agar with an attractant gradient.

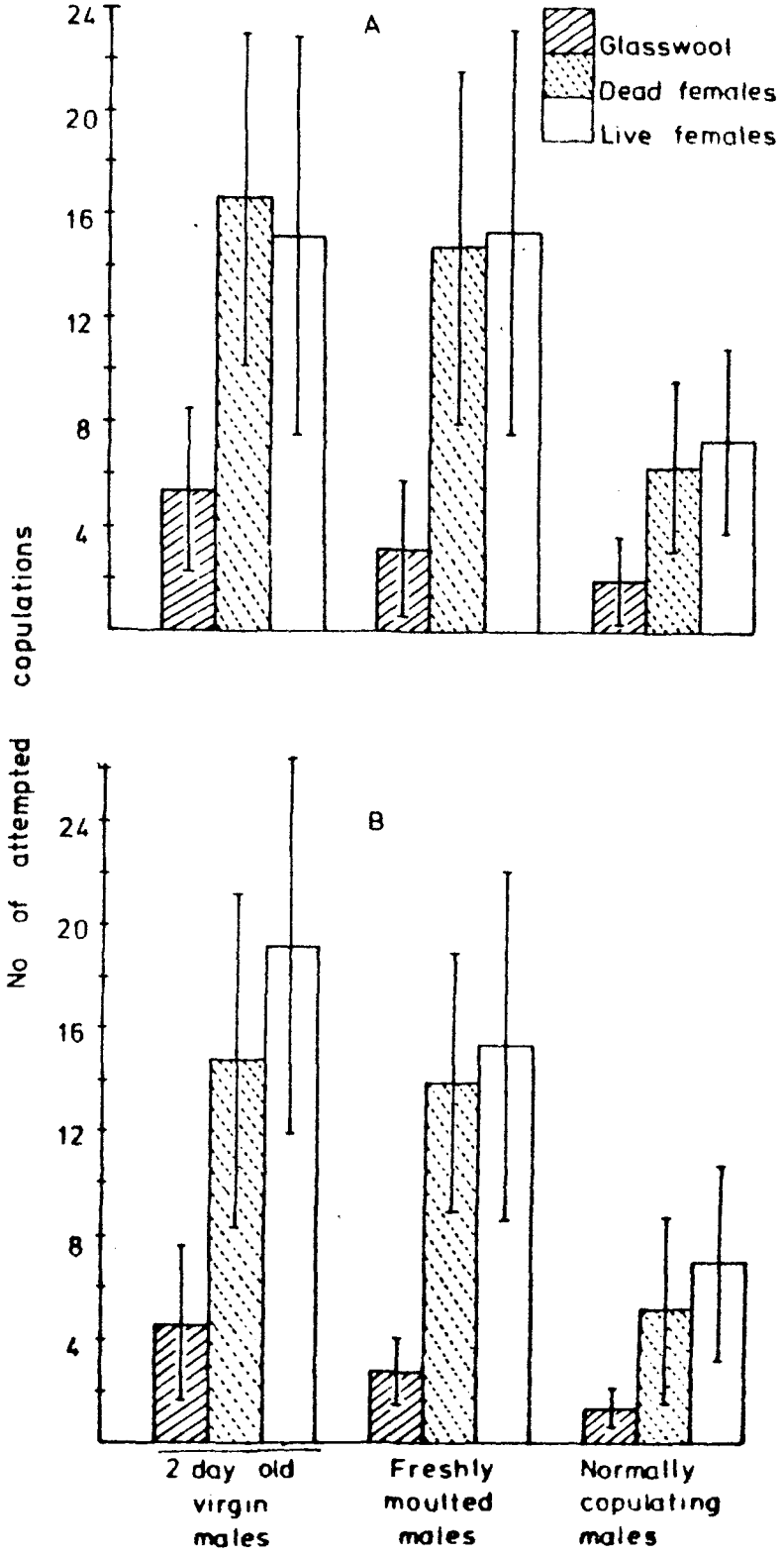

A
B
Glasswool
Dead females
Live females
No of attempted copulations
24
20
16
12
8
4
2 day old virgin males
Freshly moulted males
Normally copulating males

FIG. 55

Freshly moulted males: These males also showed a greater preference towards dead and live females than towards glasswool ($P=<0.05$; $P=<0.025$ respectively) but between dead and live females there was no significant difference ($P=>0.1$). The copulatory attempts made on glasswool, dead or live females were not significantly different between two day old virgin males and freshly moulted males ($P=>0.1$).

Normally copulating males: Differences in copulatory attempts between glasswool and live females and glasswool and dead females was significant ($P=<0.025$; $P=<0.05$ respectively) but not so between dead and live females ($P=>0.1$). The differences in attempts on glasswool made by two day old males and normally copulating males was significant ($P=<0.05$) but between freshly moulted males insignificant ($P=>0.1$). On dead females there was no significant difference between the above groups ($P=>0.05$) and on live females also there was no significant difference ($P=>0.05$).

Copulatory pattern

Freshly moulted males: The number of copulations per day decreased as the males grew old. Normally copulating males generally showed maximum copulations on the second day while on the third day the intervals between successive copulations was

greater than on the second day (Fig. 36). The maximum number of sperm per copulation was transferred on the first day (first copulation) while the minimum usually occurred on the second day. Further, the lesser the time interval between successive copulations, smaller were the number of sperm transferred (Fig. 36).

Two day old virgin males: The general pattern of sperm transfer showed a gradual decline (Fig.37). In contrast to normally copulating males, two day old virgin males showed maximum number of copulations on the first day and the mean number of sperm transferred per copulation on this day was consistently more than the average number transferred per copulation during its entire life span. After 24 hr the intervals between successive copulations was almost equal while on the first day the intervals were very short.

Alternate copulating and isolated females: Males isolated for alternating periods of 14 hr showed a unique copulatory pattern (Fig. 38). In the 10 hr period in which the males were released with females, as many as five and a minimum of four copulations took place. The maximum period between copulation was 3 hr and the minimum 1 hr. The total number of sperm transferred generally decreased from the first ten-hour period to the third ten-hour period.

FIG. 36

Copulatory pattern and the number of sperm transferred by freshly moulted males over a three day period.

Each vertical line represents a single copulation

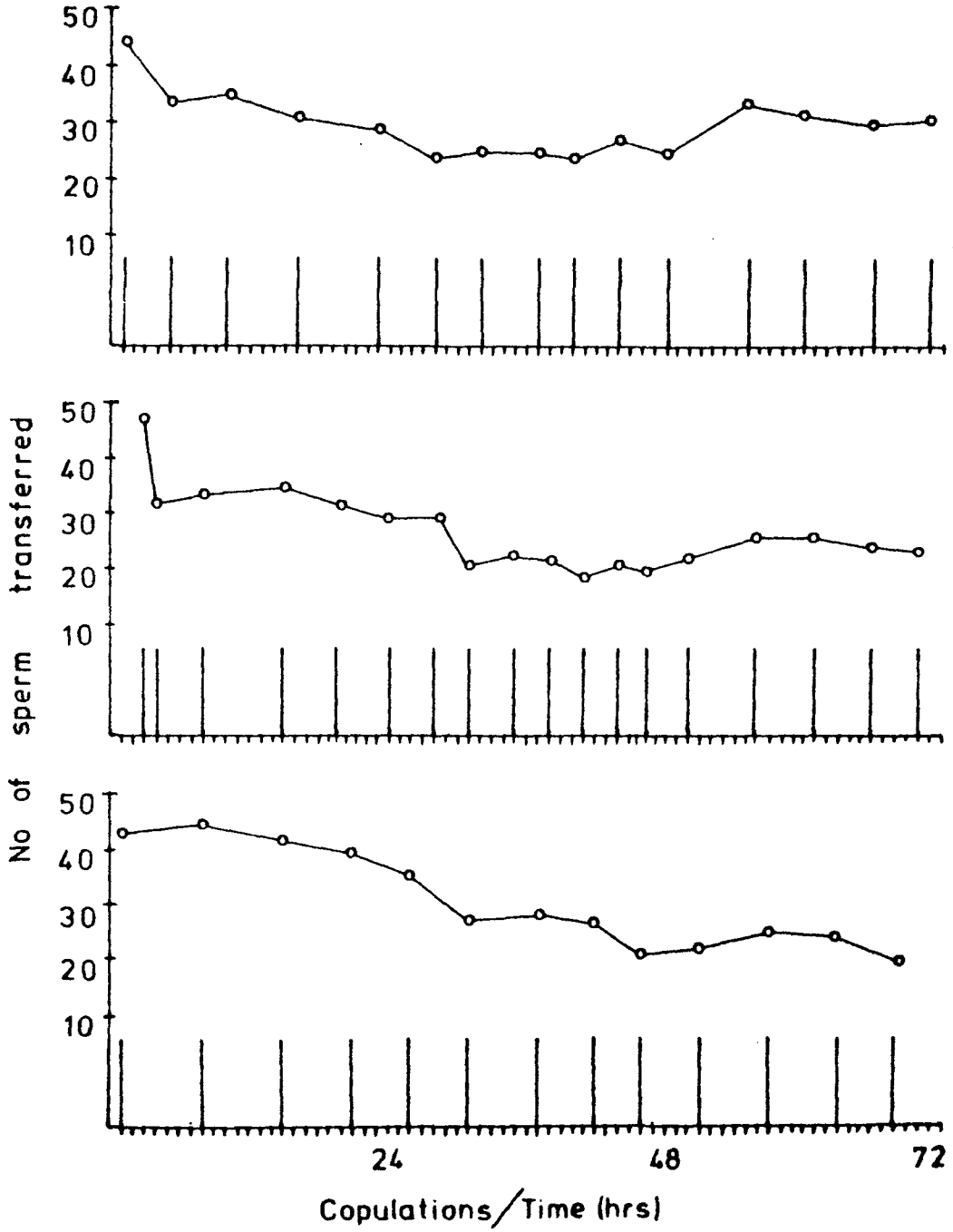

50
40
30
20
10
50
40
30
20
10
No of sperm transferred
50
40
30
20
10
24
48
72
Copulations/Time (hrs)

FIG. 36

FIG. 37

Copulatory pattern and the number of sperm transferred by two day old virgin males over a three day period.

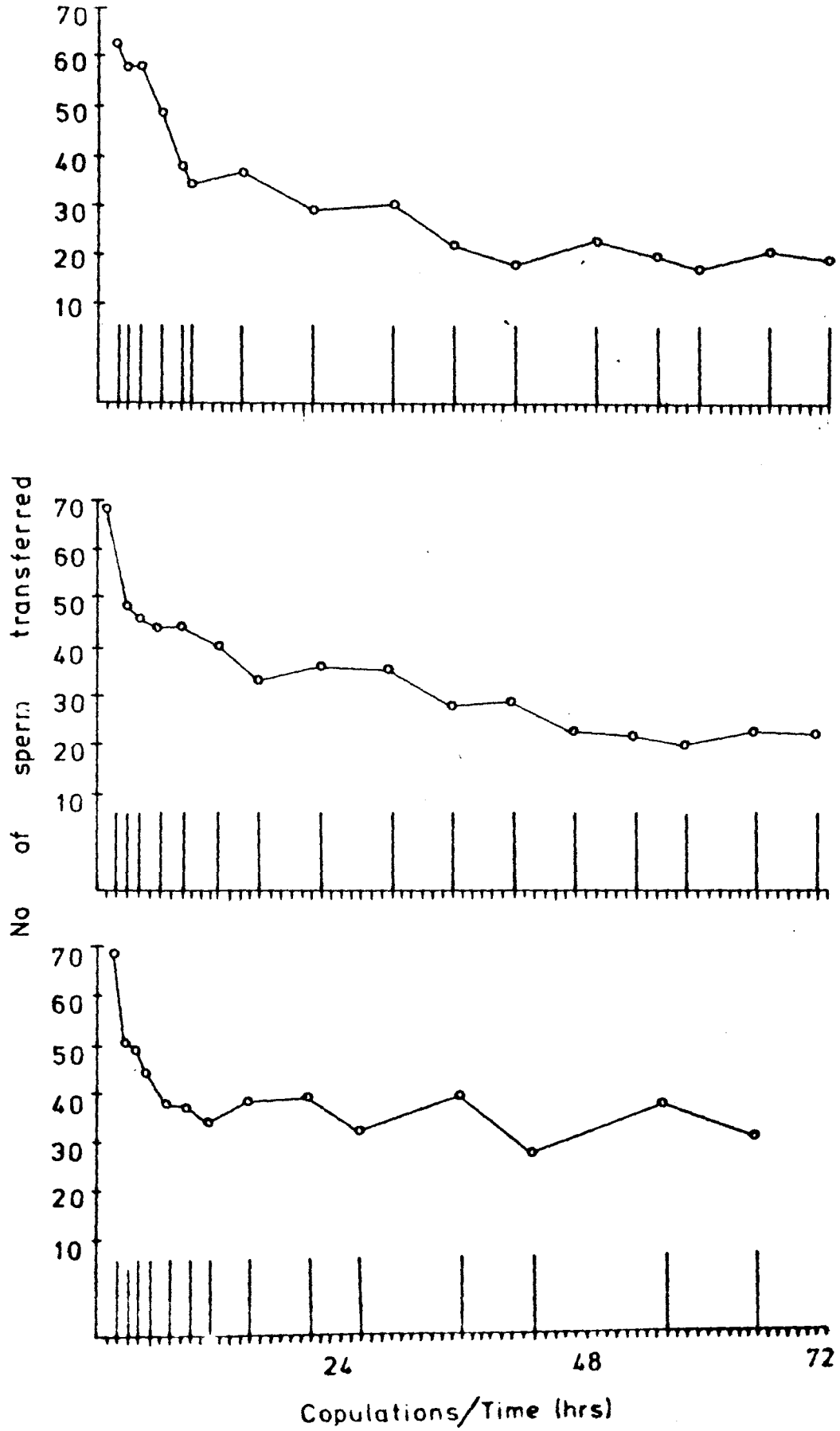

70
60
50
40
30
20
10
No of sperm transferred
24
48
72
Copulations/Time (hrs)

FIG. 37

FIG. 38

Copulatory pattern and the number of sperm transferred by males isolated for alternating periods of 14 hr over a three day period.

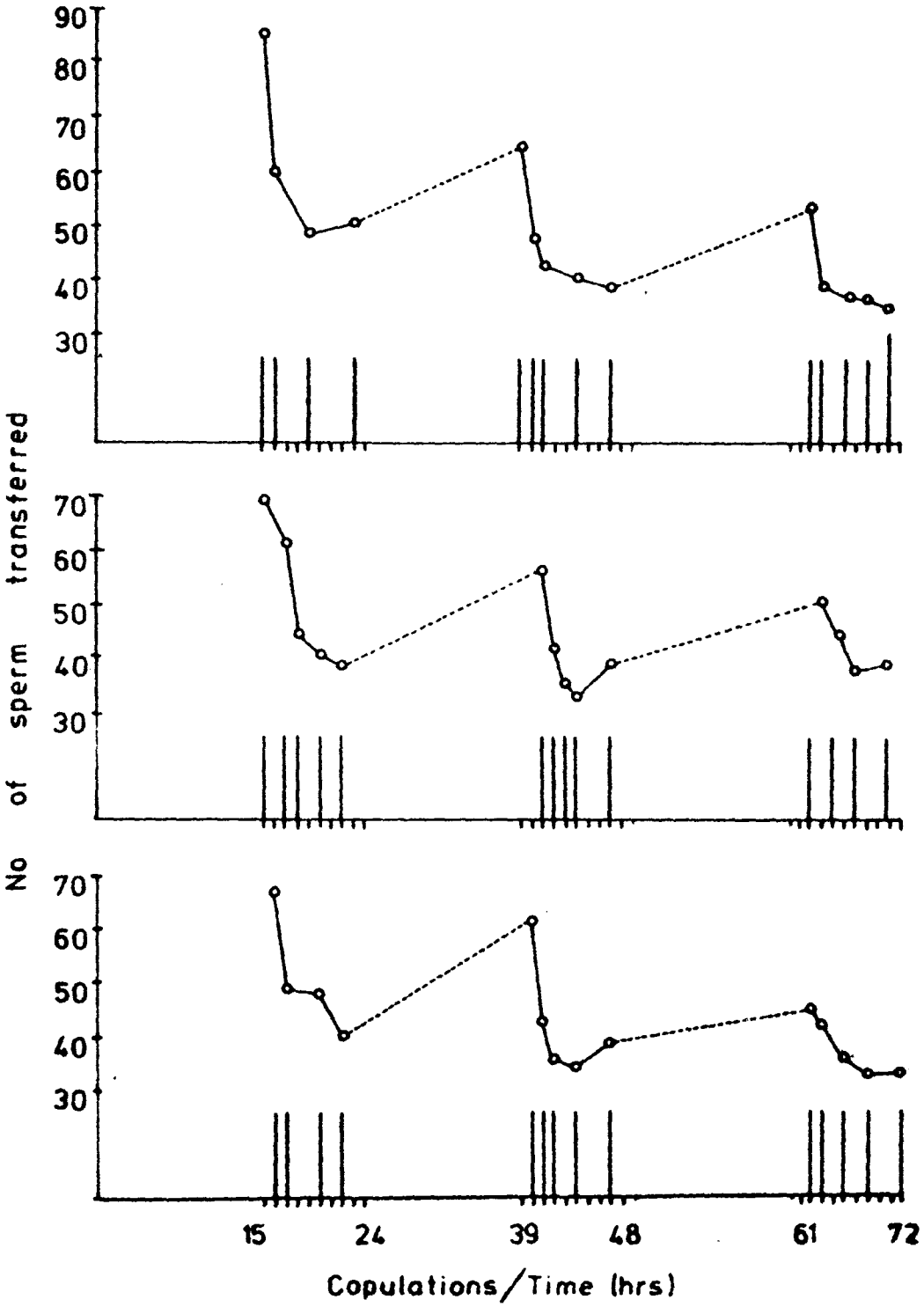

No of sperm transferred
90
80
70
60
50
40
30
70
60
50
40
30
70
60
50
40
30
15
24
39
48
61
72
Copulations/Time (hrs)

FIG. 38

DISCUSSION

On the basis of the evidence presented here, it seems that the males of Curznema lambdiensis could distinguish between a nematode (dead or live) and an inanimate object (glasswool). Although an all or none response of males towards glasswool would have been a more reliable index of discrimination, the fact that fewer attempts were made on glasswool than with dead or live females may be adequate for such a positive conclusion. The role of the sense organs and their probable functional significance is, however, obscure as no analysis was made between the point of contact with the glasswool and the subsequent behavioural response. However, when Caenorhabditis elegans contacted a bead, it withdrew a short distance and then proceeded forward in a new direction (Croll, 1976). He (l.c.) concluded that the withdrawal response was not mediated through the anterior sense organs or peripheral sensation but was under endogenous control. It is most probable that discrimination either due to a head-on collision or a glancing contact with the glasswool is under nervous control. The soft nature of the dead or live females and the rigid surface of the glasswool mediating different responses. It is proposed that accumulation of sperm in the seminal vesicle caused an endogenous sensation 'to copulate' and that under extreme conditions this endogenous system overrides the exogenous system. This may perhaps

FIG. 39

A schematic representation of the reproductive behaviour activities of *Curznema lambdiensis*.

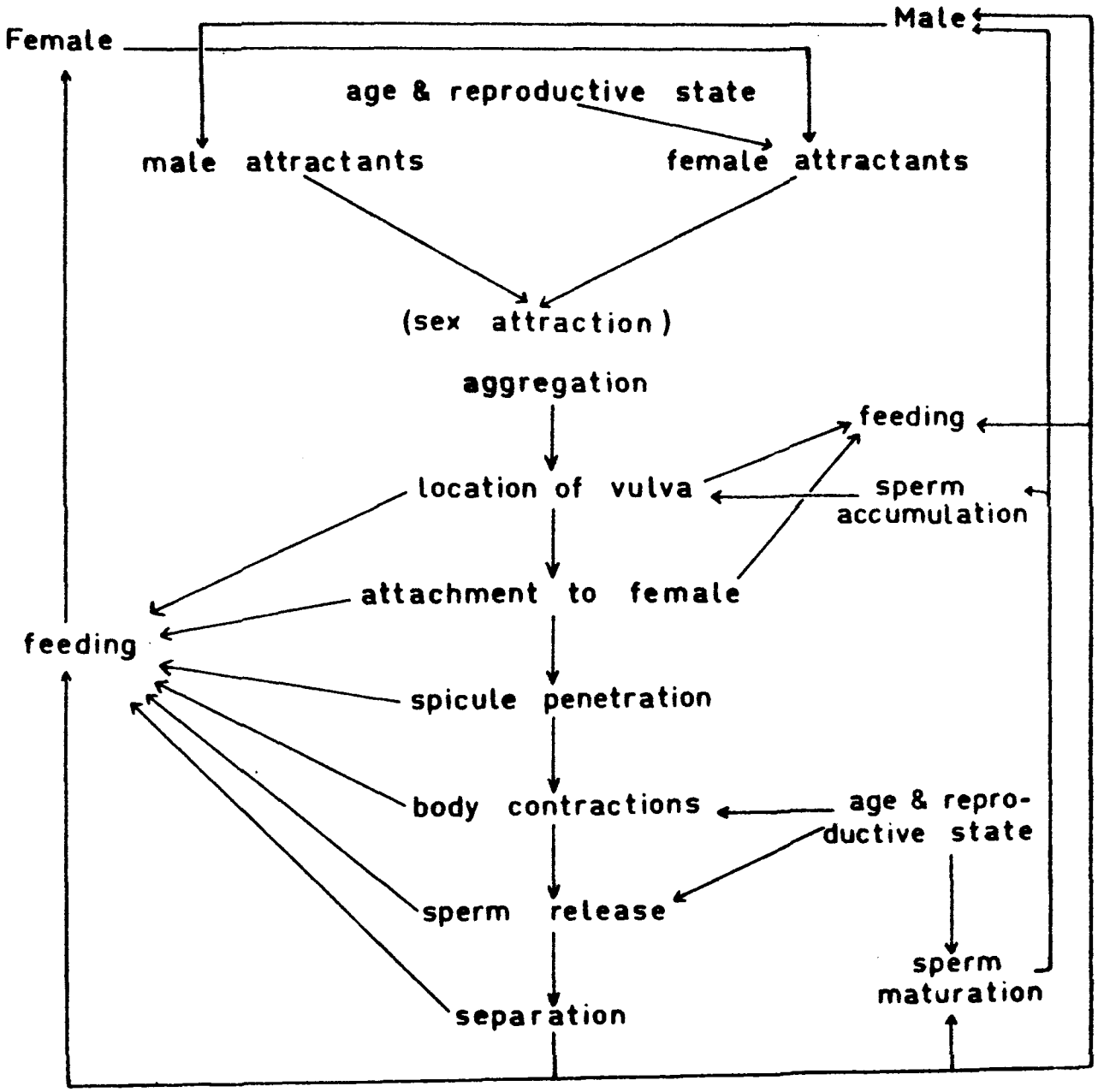

Female
Male
age & reproductive state
male attractants
female attractants
(sex attraction)
aggregation
feeding
location of vulva
sperm accumulation
attachment to female
feeding
spicule penetration
body contractions
age & reproductive state
sperm release
sperm maturation
separation

FIG. 39

be the reason why males isolated for two days attempted copulation with glasswool significantly more times than normally copulating males. Further, the behaviour of isolated males may also be explained on the basis of this endogenous sensation, the short period of rapidly occurring copulations being a consequence of accumulated sperm in the seminal vesicle of isolated males. Similarly, in the alternately isolated males, during the period of ten hours when they were released with females, hardly two or three copulations would occur in the normal conditions, but the result is on the contrary to this. Hence, it is significant that besides the greater frequency of copulations, there was also a greater rate of sperm transfer.

Sex attracting substances apparently do not influence copulation in C. lambdiensis and this may probably be true for other species of nematodes also. They only enhance the chances of copulation by bringing the sexes together. Thus males may be attracted to a potential mate but copulation may not necessarily occur. Fig. 39 gives a schematic representation of the coordination of the behavioural activities during copulation in C. lambdiensis. It is emphasized that the chief factor responsible for initiation of copulation is sperm accumulation in the seminal vesicle which in turn is dependant on the age and reproductive states on the males.

SEX ORIENTATION BEHAVIOUR OF MALE RHABDITIS SP.

Behavioural responses of nematodes may be kinesis (non-directional) as in the temperature response of Globodera rostochiensis larvae (Rode, 1970) and the light responses of Trichonema larvae (Croll, 1965; 1966) or taxes (directional) as in the orientation of Ditylenchus dipsaci to CO_2 gradients (Klingler, 1963), the response of G. rostochiensis and Heterodera schachtii to sex attractants (Green, 1966) and the response of of Panagrellus sp. to electrical stimulation and Chromadorina viridis to light stimulation (Groll, 1967a). The amphids are supposed to be chemoreceptors (Bird, 1966; Ward, 1976). Croll (1967) found that during movement these structures are at right angles to the plane of locomotion. Under these circumstances simultaneous comparison (tropotaxes) between the amphids would not be possible. When orienting to a chemical gradient Caenorhabditis elegans provided evidence of klinotaxes. Klingler (1963) suggested that D. dipsaci accumulated at CO_2 gradients by klinotaxes while Green (1966) provided evidence of both klinokinesis and klinotaxes in the responsive males of G. rostochiensis and H. schachtii. Blake (1962) attributed accumulation of D. dipsaci at host roots to klinokinesis. After observing the behaviour of several mutants of C. elegans, Ward (1976) concluded that detection of the direction of the chemical gradient was by successive sampling of the

environment during movement (klinotaxes).

MATERIALS AND METHODS

Attraction and movement patterns were observed on 2% water agar. To study tracks on non-attractant agar, the method of Croll (1975) was used but when studying tracks of males responding to female sex attractants a thicker layer of agar was used. It was first left in a thermostat to let the surface dry.

Test for random movement: For random movement the method used by Croll & Smith (1972) was utilized. A Petri dish 12 cm in diam containing 2% water agar was divided into six areas by drawing concentric circles. The central circle was 1 cm in diam and the rest were 1 cm apart. Ten males were placed at the centre of the Petri dish and their distribution was recorded after five and fifteen min. Each set was replicated five times and the pooled data of 50 males was used. The correlation coefficient between log (1- PRT) where P is the proportion of males in each circle, R is the radius of the circle and T is the time (sec), and R^2/T was calculated. A high correlation coefficient means random distribution.

In the second test, an attractant source consisting of ten young virgin females was placed in the second circle. The females

were kept in a plastic straw pipe containing agar and were incubated for 15 hr. Before observing the distribution of the males, the straw pipe containing the females was removed. The distribution of the males was observed after fifteen min.

Attraction to a point source: A fine hole was drilled in the centre of a plastic disc 1 mm thick and 5 mm in diam. Water agar was squeezed into the hole and the disc was placed in the centre of a Petri dish containing 2% water agar. On top of the disc, a plastic straw pipe of the same diam and containing ten young virgin females was placed and left for 15 hr. Before observing the tracks formed during attraction of the males, the location of the point of the attractant was marked at the bottom of the dish, and the plastic disc and straw pipe were removed. Tracks were drawn with the help of camera lucida.

Attraction to a source 2 mm in diam: A 2 mm attractant source was obtained by placing ten young virgin females in a plastic straw pipe 2 mm in diam containing 2% water agar. A small piece of filter paper was glued to the bottom of the pipe and it was placed in the centre of a Petri dish containing 2% water agar. After 15 hr incubation, the area of the attractant was marked at the bottom of the dish and the straw pipe was removed. Males were tracked individually.

Movement patterns from the centre of an attractant area: A 1 cm diam circle was marked at the bottom of a 5.5 cm Petri dish containing 2% water agar. Point attractant sources were created by placing ten young virgin females in a plastic straw pipe placed on a plastic disc with a fine hole in the centre. There were five attractant sources placed equidistantly along the circumference of the circle. After 15 hr incubation the plastic straw pipes and discs were removed and their points marked at the bottom of the dish. Males were released at the centre of the circle and tracked individually.

Gradient preference: Movement of males towards varying concentration of sex attractants were studied in a similar manner as in the above experiment except that there were only three attractant sources formed by placing one, five and ten young virgin females in plastic straw pipes. One pipe was left blank. All females were incubated for 24 hr and before releasing the males the discs and pipes were removed and their locations marked at the bottom of the dish. There were five replicates using 40 males in each.

Movement on plain and attractant agar: A thin layer of 2% water agar was poured in a 5.5 cm Petri dish. After gelation, a 1/2 cm diam block was left and the rest of the agar was removed.

Three plastic straw pipes containing ten young virgin females were placed on the agar and left for 15 hr. Afterwards, the pipes along with the females were removed and cool molten agar was poured into the Petri dish so that a continuous layer was formed. Care was taken not to let the agar form a layer over the attractant area but only be continuous with it. Hence, the attractant area would be delineated and the males would either in the attractant area or non-attractive area. All observations were made within 30 min. Males were placed at various points on the agar and tracked and timed individually.

RESULTS

Test for random movement: Movement of males on plain sterile agar was random (Fig. 40). Correlation coefficients of plotted values after five and fifteen min were significant ($P=<0.01$ and $P=<0.05$ respectively). When an attractant source was placed in the second circle, the distribution of the males after fifteen min was not random ($P=>0.1$). More males tended to accumulate in the attractant circle than in others. The resultant coefficient of correlation was insignificant ($P=>0.1$) and hence a linear regression equation was not possible.

FIG. 40

Dispersal patterns of Rhabditis sp males: ■—■ after 5 min. r= 0.9895, P= <0.001; y= 2.2862 - 9.2902x; ●—● after 15 min. r= 0.8956, P= <0.05, y= 2.539 + 24.17x; ×—× after 15 min in presence of female secretions, r= 0.6721, P= >0.1.

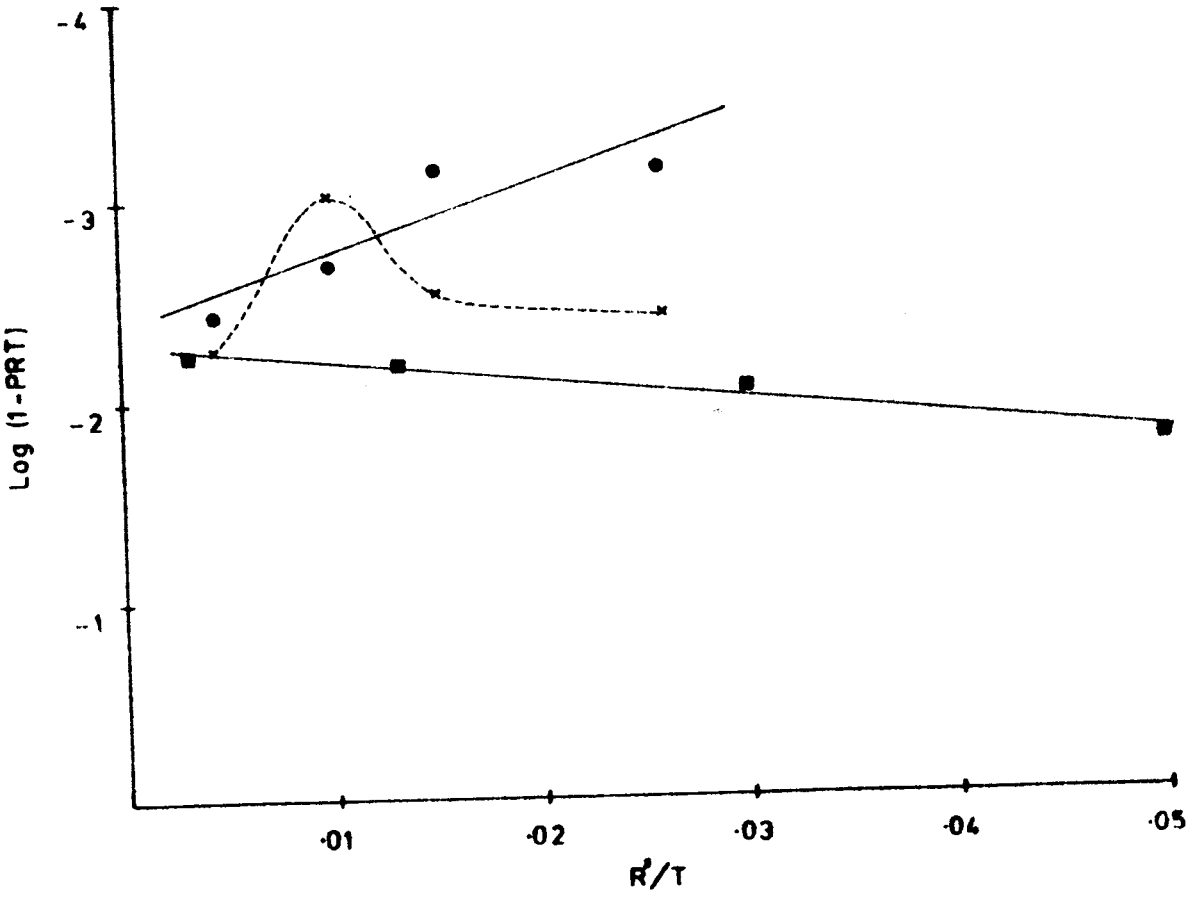


FIG. 40

Attraction to a point source: The initial response of males when placed on the surface of agar was either circular movements (Fig. 41 A) or there were a series of turns and reversals (Fig. 41 B,C). The immediate movement thereafter apparently occurred in a random direction without any indication of orientation. Such a behaviour was perhaps the result of a shock response caused by handling. Movement towards the attractant was never straight or direct and the males either followed a slightly tangential path (Fig. 41 A,B) or took a very tortuous one (Fig. 41 C). In the former case, the males after moving away from the attractant for a short distance reversed and then moved up the gradient. In the latter the male, never moved towards the attractant but did pass a point where it was nearer the attractant than initially. However, this advantage of non-directional movement did not seem to aid in locating the attractant and the male moved away further but later compensated its undirectional movements with a series of turns and loops and eventually reached the attracting source. While a path intermediate between the above two was more frequently taken by responding males, they were never observed moving directly up to the point of attraction from more than a distance of 1.5 mm.

Attraction to a source 2 mm in diam: Movement of males to a large attractant source appeared to be more directional and

FIG. 41

Tracks of males approaching point attractant sources: a - A slightly tangential approach after initially moving away; b,c - Paths showing an indirect approach and more convoluted tracks.
A = attractant source, S = starting point.

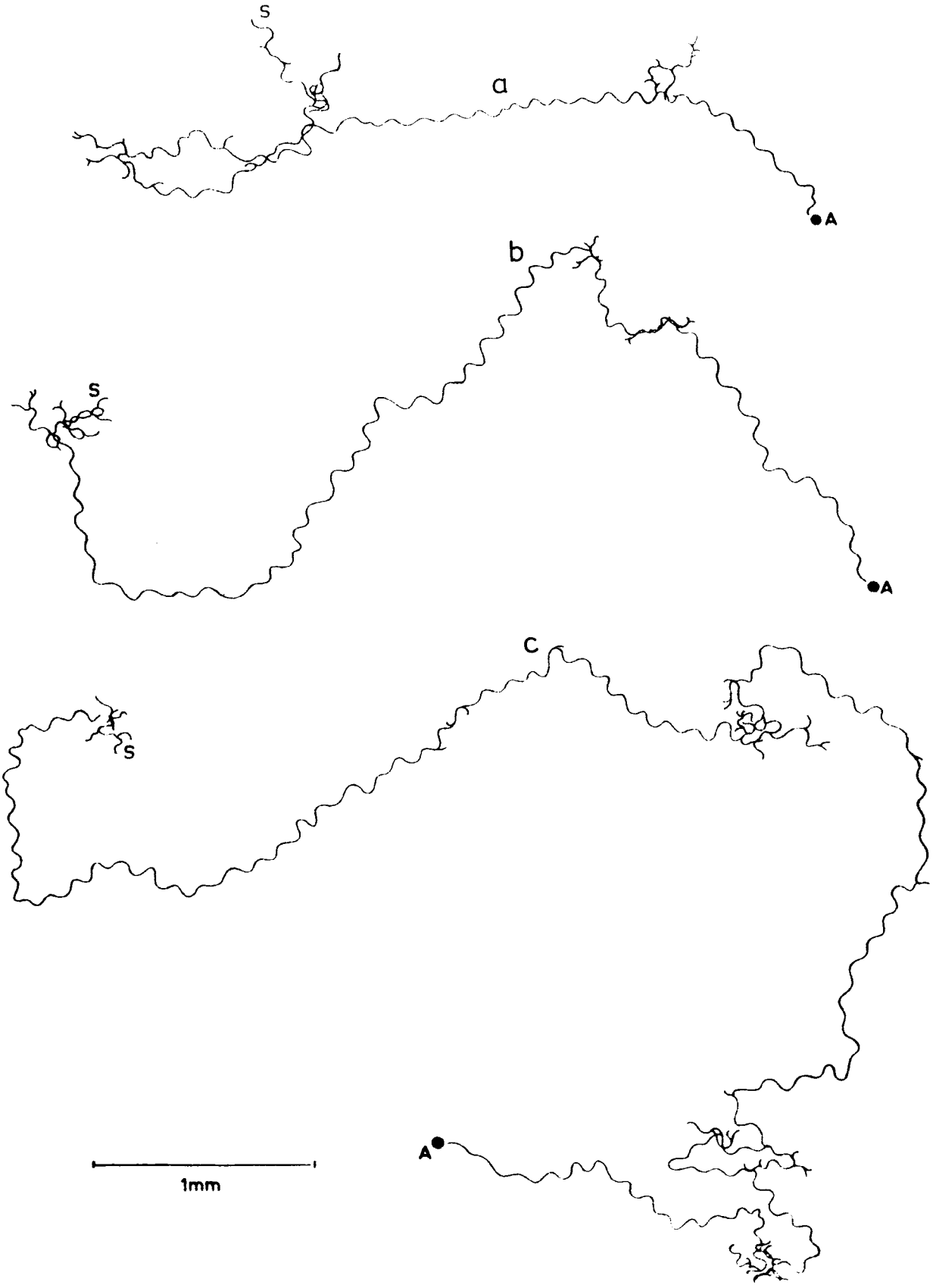


FIG. 41

FIG. 42

Tracks of males approaching an attractant source 2 mm in diam:

a - direct approach; b,c - slightly indirect approach.

A = attractant source, S = starting point.

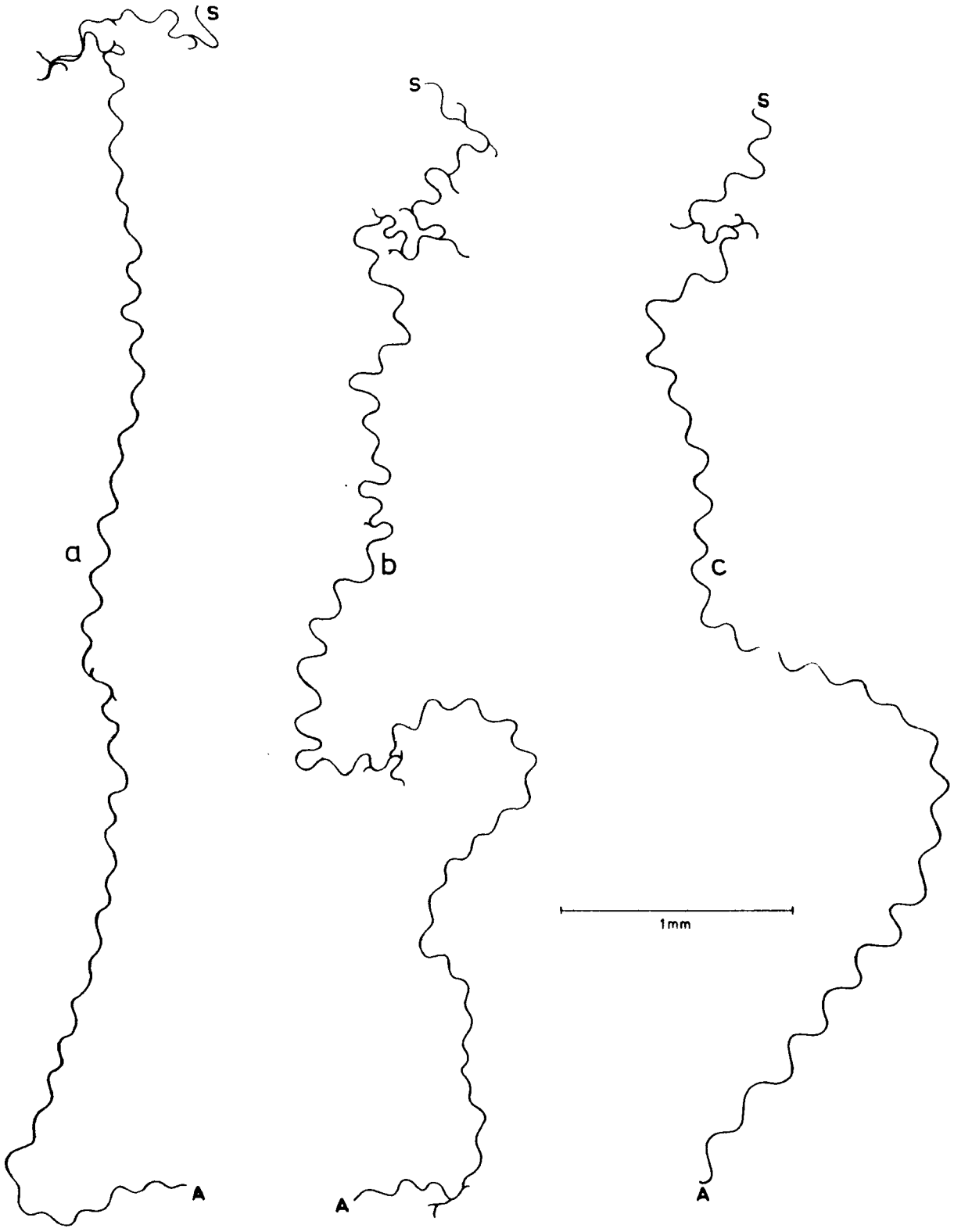


FIG. 42

less random (Fig. 42), than males responding to a point source. Males showed fewer turning and reversals and displayed a greater degree of symmetrical movement. The orientation of the body axis in relation to the attractant indicated a klinotactic response (Fig. 42 A), but in some instances, there were indications of klinokinesis (Fig. 42 B,C) even when the male was close to the attractant. After locating the attractant, males failed to move away and remained there for long periods. Often, if a second male arrived at that point and made contact with the first, both became activated and attempted to copulate with one another.

Movement patterns from the centre of an attractant area: Attractants emitted from a point source form gradients decreasing logarithmically (Green et al., 1970; Croll & Smith, 1972). At the centre of the circle, the boundaries of the attractants would merge. The expected movement of the males in the centre of the circle would be random until they moved close enough to orient to the attracting source directly. Like in the previous cases, the males when placed on the agar surface showed a complex behaviour such as numerous turns and reversals. The subsequent movement was not towards any particular direction and in most cases the body axis of the males did not point to any attractant source but somewhere in between them (Fig. 43,44). The males showed very

FIG. 43

Tracks of male moving from the centre of an attractant area showing an exteremely convoluted path in the region between two attracting sources. A = attractant sources, S = starting point.

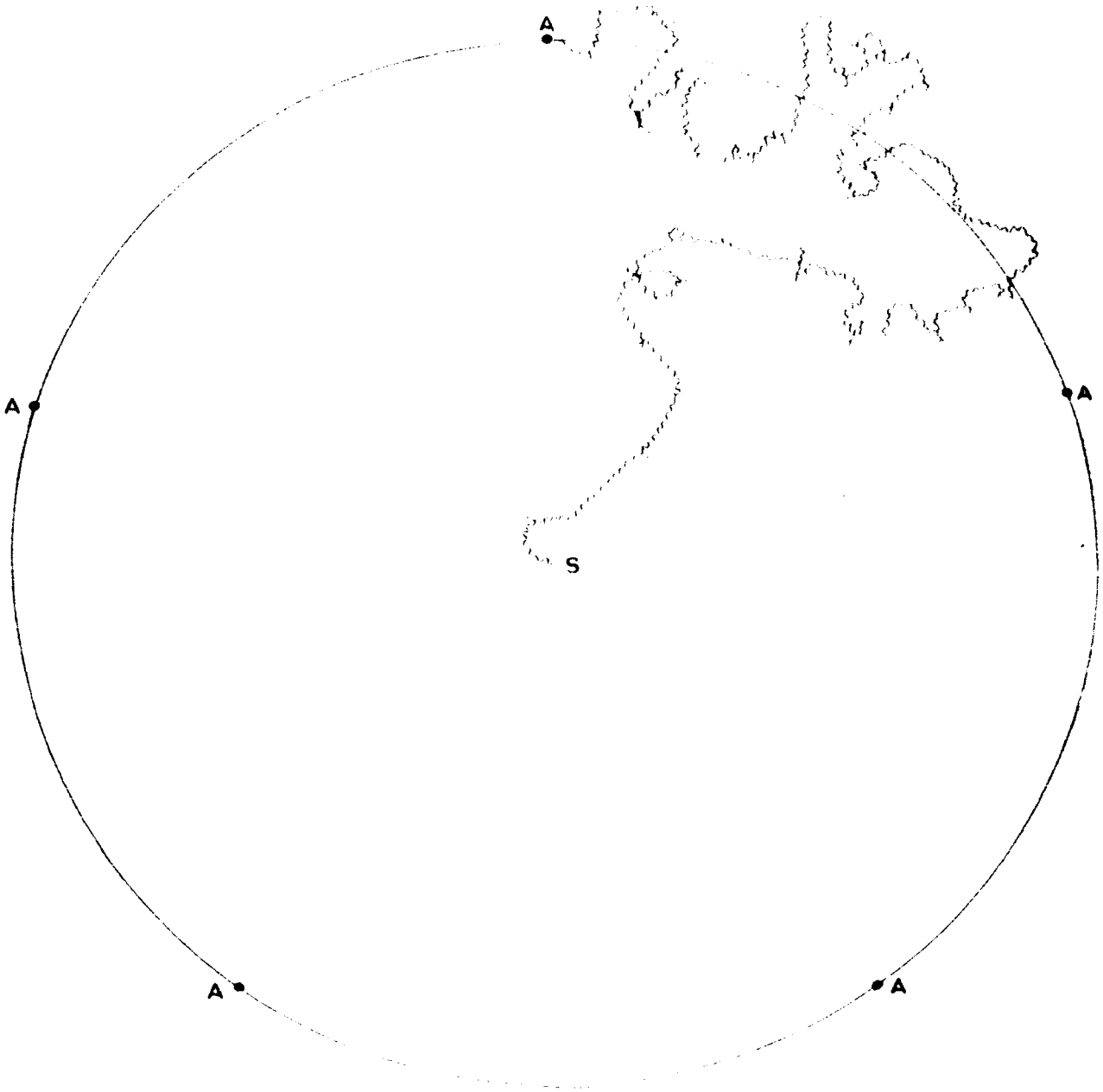


FIG. 43

FIG. 44

Tracks of a male making a more direct approach from the centre of an attractant area.
A = attractant sources, S = starting point.

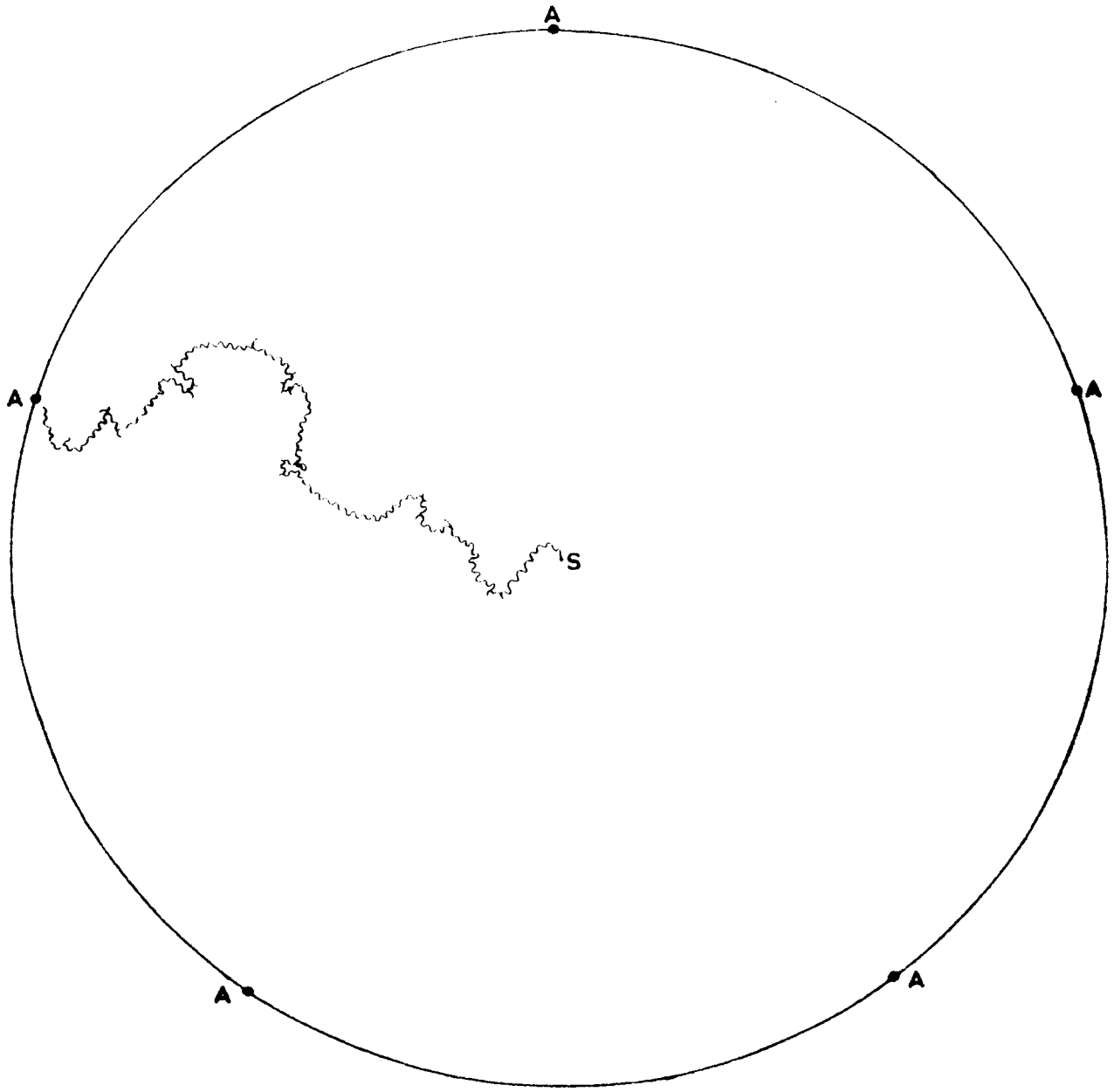


FIG. 44

FIG. 45

Tracks of a male showing circling movements and numerous turns and reversals while responding from the centre of an attractant area.
A = attractant sources, S = starting point.

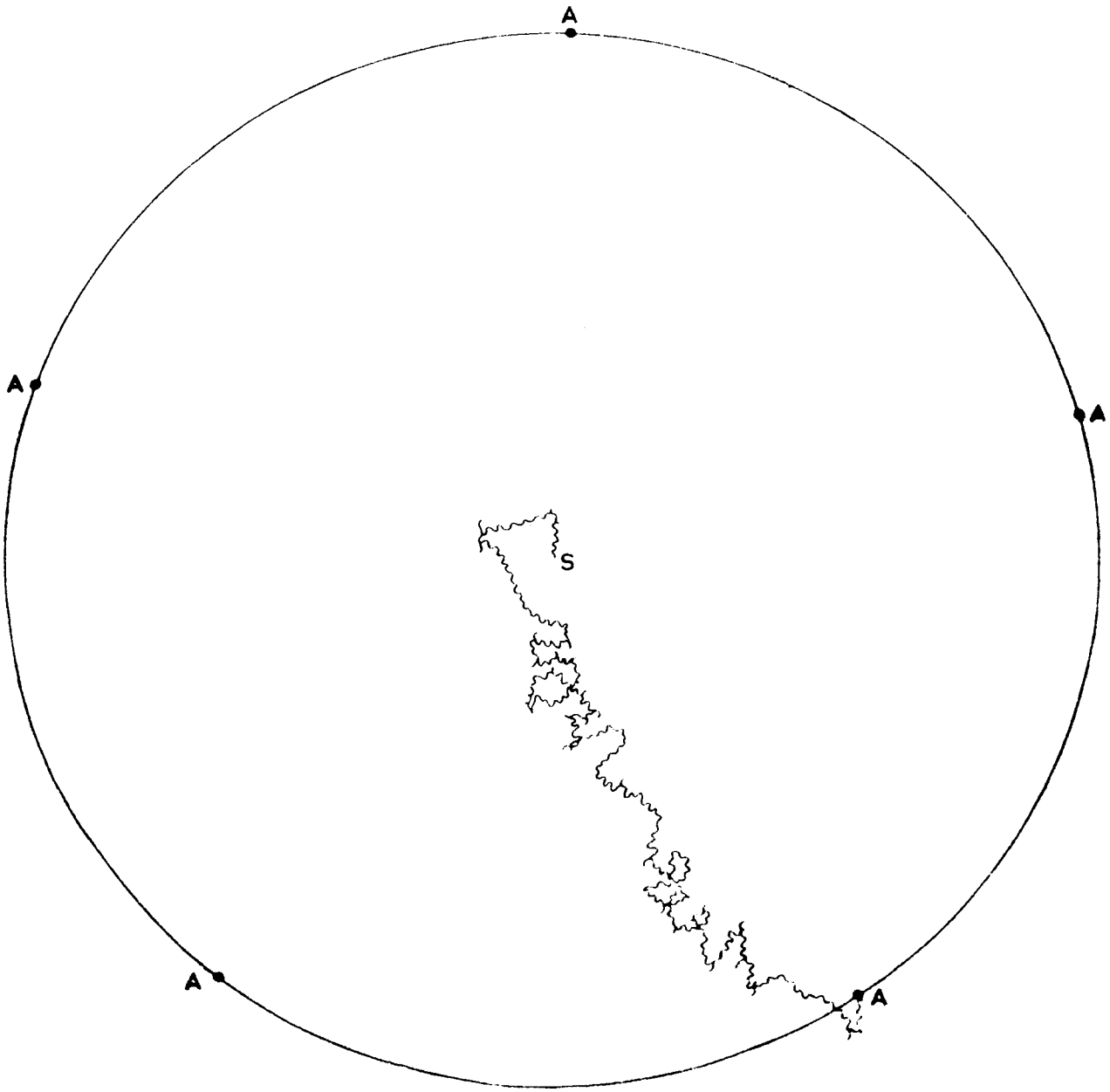


FIG. 45

convoluted tracks if they moved into the area between two attracting points on the periphery of the circle (Fig. 43). In this area steep gradients occurred only very close to the source as responding males often reversed and moved away after coming to within 2 mm of the attractant. However, males making a more direct approach had less difficulty in locating the attractant (Fig. 44). In a few cases the males showed a series of circling movements, reversals and loops (Fig. 45). This type of behaviour was of inconsistant occurrence and resulted perhaps from abnormal endogenous causes. The majority of the males tested followed a path similar to Fig. 44 but occasionally some moved out of the attracting area and did not seem to respond. Only three of the fifty males tested showed this kind of behaviour.

Gradient preference: When a varying number of virgin females were incubated for a fixed time, the attractant gradients formed would vary according to the number of females present at each point, being stronger towards more females and weeker towards fewer females. The results showed that maximum number of males migrated towards the five female source (Fig. 46). Significantly fewer went towards one and ten female sources ($P = < 0.001$). Some males also approached the blank source. The distribution of the males on the second trial was strikingly similar to the first pattern, there being no significant differences towards any

attractant ($P=>0.1$). However, the percentage of males showing the same kind of behaviour on both the trials was not more than 50% towards any of the groups of attractants. On an average, slightly less than 50% of the males migrating to the five female source on the first trial returned to the same attracting source on the second trial. The percentage of males showing similar behaviour towards the one female source was significantly reduced to 13% ($P=<0.01$). It was further reduced to 12% towards the ten female source while towards the blank source it was totally absent.

Movement on plain and attractant agar: (Fig. 47) Males moving into an attractant area showed more turning than when moving on non-attractant areas. Tracks were more convoluted in the former than in the latter. The duration of stay in the attractant area was highly variable and while some of the males moved out after only five min, others remained for well over 2 hr. Analysis of the locomotory characteristics showed significant changes in the wave length ($P=<0.01$) in the attractant and non-attractant areas. While in the attractant area the wave length decreased but the amplitudes of the waves remained constant in both the areas. The wave frequency decreased when the males entered the attractant area suggesting a decrease in the activity.

FIG. 46

Responses of males to attractant sources formed by a varying number of females.

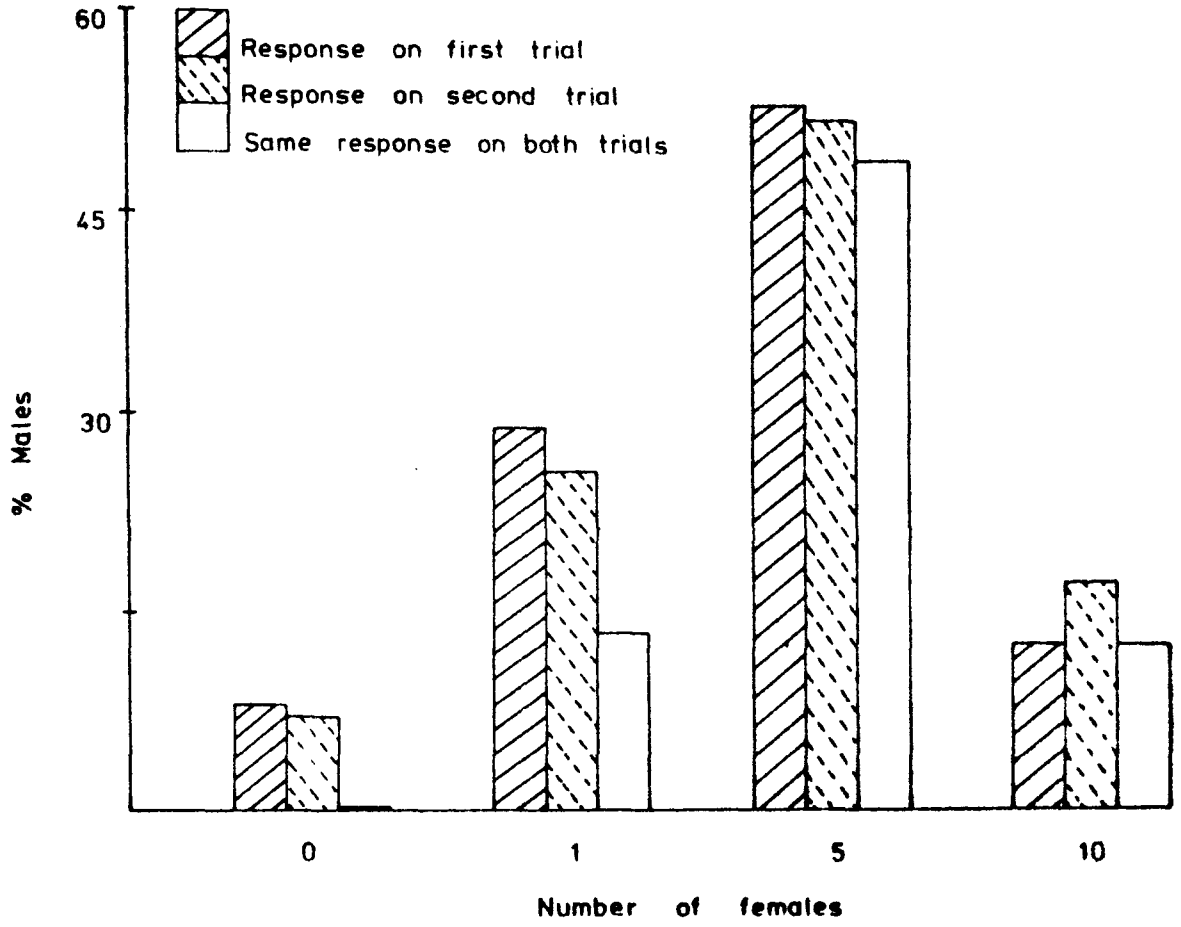

60
45
30
% Males
Response on first trial
Response on second trial
Same response on both trials
0
1
5
10
Number of females

FIG. 46

FIG. 47

Tracks showing the movement patterns of three males in an attractant and non-attractant area. Arrows indicate the direction of movement and the circle represents the attractant area.

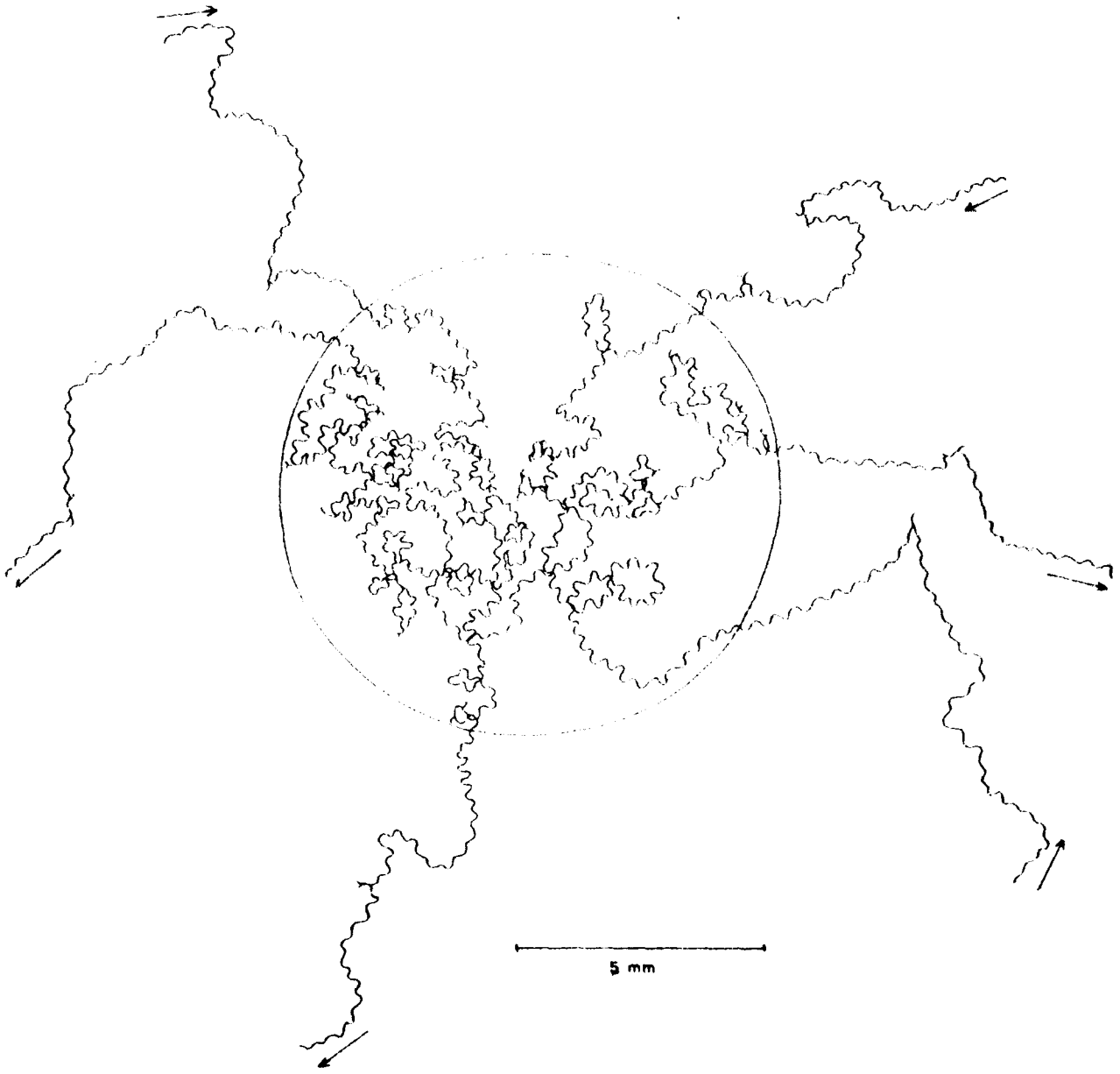


FIG. 47

TABLE III

Wave characteristics in attractant and non-attractant areas

Wave characteristics	Medium: Attractant	Medium: Non-attractant
Wave length (um)	252.4	386.2
Amplitude (um)	56	56.4
Wave frequency (waves/min)	42.8	88.8

DISCUSSION

By the random walk test it was evident that on plain sterile agar males of Rhabditis sp. moved in a random manner. Non-random distribution of the males in the presence of female secretions indicated the occurrence of male attracting substances produced by females as is already known in some other nematode species (Green, 1966; Jones, 1968). These sex attractants not only attracted males and retained them but also caused a decrease in their activity, which was measured in terms of number of waves formed by the males per unit time.

Males responding to female secretions showed a variety of responses depending on the nature of the attracting source. Attractants eminating from a point source form gradients decreasing logarithmically and have steep gradient only very close to the point of attraction (Greenet al., 1970; Croll & Smith, 1972) while gradients formed from a large source presumably have steep gradients spread over a larger area. The apparent differences in orientation of males towards these two kinds of sources thus arise from the different gradient build up. Green (1966) concluded that males of G. rostochiensis and H. schachtii respond to sex attractants by klinokinesis and klinotaxes. In C. symmetricus orientation involves klinokinesis and klinotaxes alternately.

Besides the initial shock responses, the basic orientation behaviour of Rhabditis sp males involved klinokinesis. However, the final approach path to the attractant which was in straight lines may be significant from the point of view that closer to the attractant the gradient would be very steep. In addition, reaction to multidirectional attractants showed that if males wandered into the area at the circumference of the circle between two adjacent attractants, the tracks were highly convoluted. Again in this case, only when the males came very close to the attractant did they show a direct approach. In most cases directional locomotion was not observed even when the male was nearer to one of the attractants. This inability of directional movement when between two sources probably resulted from overlapping gradients. Ward (1976) suggested that C. elegans orientated to a chemical gradient by klinotaxes. For klinotaxes, more direct approaches should be expected. The initial undirected movement probably resulted from males being unable to distinguish the gradient. In contrast, males responding to attractants from a large source (2 mm diam) showed a more direct approach. Undirectional movements were few or none and hence orientation resulted from klinotaxes.

Samoiloff et al., (1974) interpreting the tracks of Panagrellus redivivus males responding to females suggested that conditions of no stimulation, males moved in almost straight lines

with a low amplitude, but when stimulated, they became activated showing rapid changes in behaviour and the head swinging over in a wide amplitude. These authors, however, did not analyse the wave characteristics and activity. Observations with Rhabditis sp showed similar behaviour in some respects and disimilar in others. Upon being stimulated the males showed a decreased activity by decreasing their wave frequency. The amplitude remained constant but the wave length decreased significantly, Hence the number of waves per body length increased. Increase in the change in direction, as evidenced by more turnings, may perhaps have functional significance by allowing sampling of a wider area while the decrease in activity inhibits the males from wandering away from the attractant area. It may be significant to note that while interpreting orientation with the help of tracks, the obvious conclusion is that it involves klinokinesis and/or klinotaxes but analysis of the locomotory characteristics provides evidence for an orthokinesis.

SUMMARY

In the present work an attempt was made to elaborate and explore in detail, the reproductive behaviour of some soil-inhabiting nematodes. Experiments on sex attraction, copulatory behaviour, copulatory senses, ageing and reproduction, and orientation were conducted on the following three species of nematodes: i) Chiloplacus symmetricus, ii) Curznema lambdiensis and, iii) Rhabditis sp.

Observations on sex attraction in Chiloplacus symmetricus showed that only the males were attracted to female secretions, females and fourth-stage male juveniles did not respond. Males showed no significant attraction towards male or fourth-stage female juvenile secretions. The females did not respond to either male or fourth-stage female juvenile or their own secretions. Similarly, fourth-stage male juveniles were unresponsive to any secretions. Orientation of males to sex attractants involved both klinokinesis and klinotaxes, the final approach being a direct movement. Copulation took place when the male coiled its tail around the female and the spicules located the vulval opening. The spermatozoa after deposition in the uterus moved upwards and accumulated in the spermatheca. Copulation lasted from half a minute to nearly 45 minutes.

A study of the factors influencing sex attraction in Chiloplacus symmetricus revealed that movement of males to attractant sources was highly variable depending on the experimental techniques employed. In Petri dish experiments, fewer females produced a significant male response than in the mickey mouse traps and also the incubation period needed by the females to produce a response from the males was lesser in the former experiment. Attraction was evident earlier in the Petri dish experiments than in mickey mouse traps. Within each type of experiment also, sex attraction varied with the number of females at the attractant source, period of incubation, time of observation, thickness of agar and the concentration of agar. Generally, five females incubated for 18 hr resulted in a good response of males in the Petri dish experiment but at least 50 females incubated for 18 hr were required for the mickey mouse trap. Attraction was evident towards five females in the former experiment in 2 hr and towards 50 females in the latter experiment also in 2 hr. 1,2 and 4 mm thick layers of agar did not produce any change in attraction but in 8 mm thick agar attraction decreased significantly. Agar concentration of 4 and 8% inhibited sex attraction in both sets of experiments while there was no significant difference in 1 and 2% agar. Light produced no significant difference in attraction in either experiment.

The sex attraction of ageing males and females of C. symmetricus showed that this phenomenon was dependant on the age and reproductive state of the worms. All age-groups of virgin males were responsive to all age-groups of virgin females except 22 day old males to 18 and 22 day old females. The response of young males to older females decreased gradually and similarly the response of ageing males to younger females also decreased gradually. Attraction between virgin males and non-virgin females showed that males of all age-groups were attracted to 10 day old non-virgin females and all except 22 day old males were also attracted to 14 day old females but males did not respond to older females. Males of all non-virgin age-groups showed a positive response to virgin females of all age groups except 22 day old males to 22 day old females. In non-virgin males to non-virgin females, 10 day old females were attractive to males of all age groups and only 10 and 14 day old males were attracted to 14 day old females. Females of other age-groups were not attractive.

In Curznema lambdiensis males did not attract males and similarly females did not attract females. Young virgin males responded to young virgin females but not to old virgin females. Young virgin females, however, responded to both young and old virgin males. Virgin males were not attracted to non-virgin females

but non-virgin males were attracted to virgin females. Non-virgin females showed a positive response to virgin males and virgin females also responded to non-virgin males. Attraction of males to females and females to males increased when the number of attractant worms increased to 50 from ten but a further increase did not produce a corresponding increase in attraction. When both males and females were put at the attractant source, the attraction of females increased from female: male ratio 1:50 to 20:50 but declined thereafter to increasing ratios. Males, however, did not show any similar increase to male: female ratio and attraction gradually decreased from 1:50 to 50:50 male:female ratio.

From the studies on the copulatory behaviour of Curznema lambdiensis it was concluded that copulation involved three distinct steps: i) attachment to female and location of the vulva, ii) penetration by the spicules and, iii) insemination. The bursa aided in gripping the female while the spicules located the vulval opening but did not take part in channelising the sperm from male to female reproductive tracts. The build up of internal pressure to release sperm was accomplished by shortening and swinging of the body in wide arcs. Females continued feeding during copulation. The mean number of copulation per day varied from 3 to 7.2 and the sperm transferred per day from 61 to 176. On an average 20-33 sperm were transferred per copulation per day. In its life span,

a male copulated 15 to 32 times and transferred a total of 517-754 sperm. When males were isolated for more than two days, both the number of copulations and the number of sperm transferred decreased. The mean number of sperm transferred on the first copulation was maximum in two day old males while three and four day old males showed a significant decline. As the isolation period of the males increased, the time required for the first copulation also increased.

In ageing virgin females of Curznema lambdiensis the number of oocytes released by the ovary was less than in copulating females. Unfertilized oocytes failed to develop an egg shell and usually ruptured in the uterus. The egg mass sometimes passed out of the body during vulval twitchings or was reabsorbed by the uterine walls. In old virgin females, the ovary gradually became vacuolated and then shrivelled up. In copulating females as many as 171 eggs were produced on the first day. Fertilization took place in the spermatheca but the oocytes first contacted the sperm in the oviduct. Eggs were laid in batches but in older females they were retained in the body and ultimately led to 'endotokia matricida'. Spermatozoa in virgin males began maturing by the end of the final moult and within a day filled the entire seminal vesicle. On the third day they began to degenerate. Such degenerate spermatozoa had either a condensed cytoplasm or their outer layer became mammilated. The

testis degenerated in the same way as the ovary. Normally copulating males did not accumulate sperm in their seminal vesicle. Degenerative changes started on the third day. The mean life span of virgin worms was 10 days while of non-virgins it was only 6.5 days. When ageing males were mated with young females or vice-versa, egg production gradually decreased. Similar results were obtained for egg production after the first copulation.

An analysis of the copulatory senses of C. lambdiensis revealed that males, either virgin or non-virgin, could distinguish between an inanimate and an animate object but could not differentiate between dead and live females either on sterile agar or on agar with an attractant gradient. Freshly moulted males copulated regularly over the entire three day period but maximum sperm were transferred during the first copulation. Two day old males copulated rapidly and a greater number of sperm were transferred per copulation on the first day than on the second and third day. On the second and third day the intervals between copulations increased. In alternately isolated and copulating males, copulations occurred at a faster rate than normally and the number of sperm transferred was also greater.

Movement of males of Rhabditis sp was random on plain sterile agar as was indicated by the high correlation coefficients but in the presence of female secretions, males showed a bias pattern of

movement (no correlation) and tended to accumulate at the source of attractant. Males orienting to a point source showed more turnings and asymmetric movements than when orienting to a source 2 mm in diam. In the former case, tracks were often extremely circuitous. The movement of males from the centre of an attractant area was highly variable and in most cases, the males orientated initially towards a point between two attractant sources and only when near the source did they move directly. However, those males that moved into the area between two attracting sources at the periphery of the circle, showed very tortuous tracks and were captured by an attractant source only when they came very close to it. Males responding to attractants showed preferential movement and most aggregated at the five female source on both the attempts. The maximum number of males showing the same response on both the trials was at the five female source. However, not more than 50% of the males showed the same response on both the trials. Analysis of the locomotory characteristics in attractant and non-attractant zones of agar revealed that sex attractants inactivated males i.e., decreased their wave frequency. The wave length increased but the amplitude remained constant.

APPENDIX

LOCOMOTORY CHARACTERISTICS OF *CHILOPLACUS SYMMETRICUS* JUVENILES

The locomotory behaviour of the second stage juveniles of *Meloidogyne javanica* has been described by Wallace (1969) and he found that all parameters used in defining locomotory characteristics decreased as the concentration of the agar increased except the number of waves formed per body length. Pollock & Samoiloff (1976) studying the responses of the juvenile stages of *Panagrellus redivivus* observed that each stage responded to different bacteria and concluded that behaviour was specific for each stage. In this work, the locomotory characteristics of *Chiloplacus symmetricus* juveniles was studied in different concentrations of agar.

MATERIALS AND METHODS

The locomotory characteristics of *C. symmetricus* juveniles was assessed by analysing the tracks formed on the surface of agar. Four concentrations of agar, viz., 1%, 2%, 4% and 8% were used and each juvenile stage was tracked and timed individually in all the agar concentrations. Before recording the observations, the nematodes were allowed to move about to let them habituate the medium. Tracks were drawn with the help of camera lucida. All juvenile stages were isolated directly from running cultures and

placed in water before experimentation. The various parameters analysed and ratios deduced were wave length ($\propto$), amplitude (λ), wave frequency (N), velocity along axis (V_a), pitch of wave (θ) and wave length/body length.

RESULTS AND DISCUSSION

The wave length of tracks increased with increase in body length (i.e., 1st, 2nd, 3rd and 4th stage juveniles). However, the ratio of wave length/body length was almost constant for all the stages in all concentrations of agar (Table 4) suggesting that the increase was proportionate. The wave length appears to be about half the body length in all stages. The amplitude also increased with older juvenile stages but its relationship with the wave length was not similar in all the juveniles. Thus, the pitch of waves ($\theta = \frac{\propto}{\lambda}$) shows a marginal increase with increasing concentration of agar in the first-stage juveniles. In the second and third-stage there was no general trend while in the fourth-stage θ increased greatly from 1% agar to 4% and 8%. The increase in amplitude did not cause a decrease in wave length in the first, second and third-stage juveniles but was so in the fourth-stage juveniles (Fig. 48). Hence, only in the fourth-stage juveniles, amplitude and wave length are inversely related to each other.

TABLE IV.

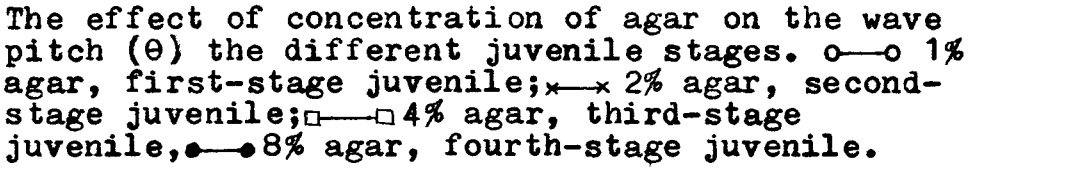
Locomotory characteristics of C. symmetricus juveniles

Wave characteristics	Juvenile stages	Concentration of agar 1%	2%	4%	8%
Wave length (um)	1st	187	153	177	172
	2nd	223	223	255	247
	3rd	248	296	268	274
	4th	329	383	302	363
Amplitude (um)	1st	22.3	27.8	25.6	26.4
	2nd	29.1	28.3	34.8	33.0
	3rd	31.0	45.7	38.0	42.0
	4th	44.0	67.2	60.5	71.0
Wave length / Body length	1st	0.522	0.496	0.472	0.467
	2nd	0.435	0.554	0.531	0.513
	3rd	0.418	0.526	0.470	0.478
	4th	0.396	0.542	0.428	0.510
Body length (um)	1st	358	308	375	368
	2nd	513	402	480	482
	3rd	592	562	570	574
	4th	830	707	705	712

The wave frequency in the first-stage juveniles varied within small limits as the concentration of the agar increased. In the second stage, the variation was greater but there was a tendency to increase N with increase in agar concentration. Third and fourth-stage juveniles showed a sharp increase in wave frequency with stiffer agar media (Fig. 49 A). A stage-wise comparison of wave frequency revealed that this component of locomotion decreased in 1% agar as nematodes advanced in their life cycle. In 2% agar wave frequency increased in the second and third-stage but showed a sharp decline in the fourth-stage. In 4% agar, the increase was greater than in 2% and the decline in the fourth-stage smaller. In 8% agar wave frequency increased continuously. From Fig. 49, A it becomes apparent that the degree of variability of wave frequency is least in the first-stage, greater in the second and third-stage and maximum in the fourth-stage. The smaller wave frequency in 1% agar of older stages may perhaps be caused by a greater percentage of slip in large nematodes than in the smaller ones while the reversed condition in 8% agar may probably be due to a greater force required in moving on stiffer medium.

The velocity of juveniles along the axis increased as the age of the nematodes increased. In the first-stage juveniles there

FIG. 48


The effect of concentration of agar on the wave pitch (θ) the different juvenile stages. o—o 1% agar, first-stage juvenile;×—× 2% agar, second-stage juvenile;□—□ 4% agar, third-stage juvenile,●—● 8% agar, fourth-stage juvenile.

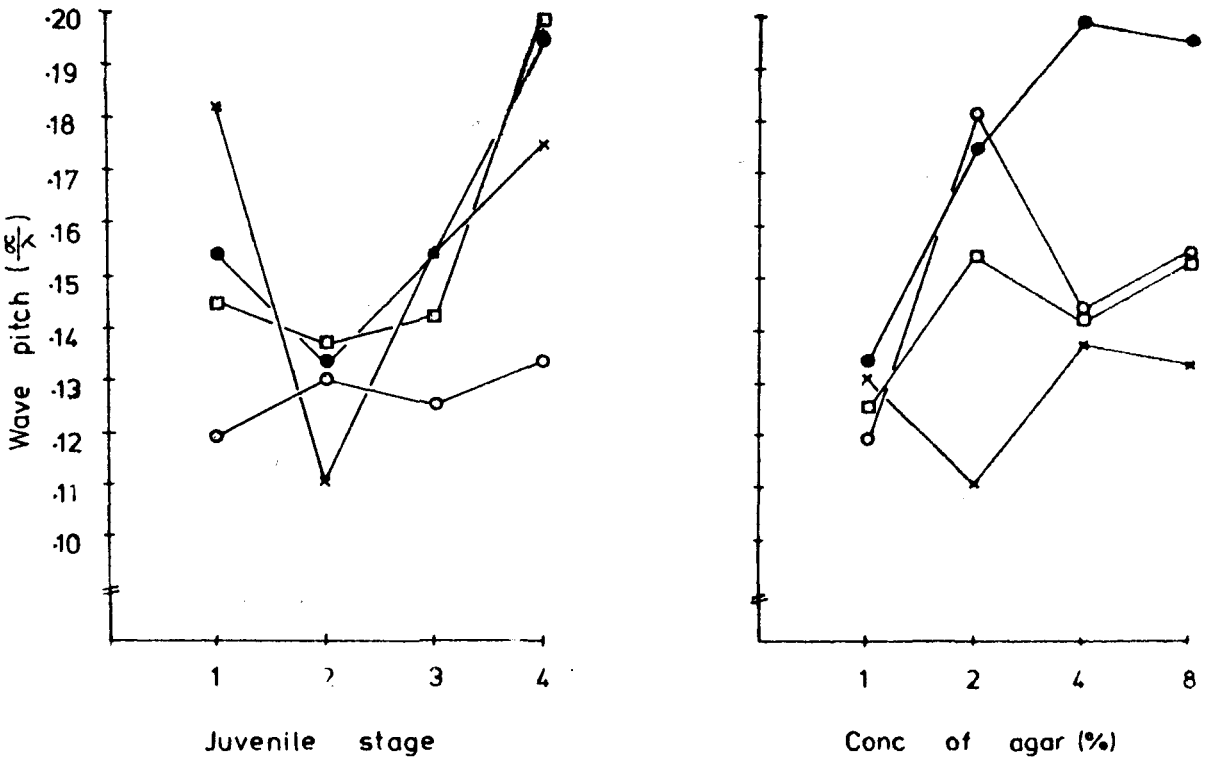

·20
·19
·18
·17
·16
·15
·14
·13
·12
·11
·10
Wave pitch (λ/x)
1
2
3
4
Juvenile stage
1
2
4
8
Conc of agar (%)

FIG. 48

FIG. 49

A - The effect of the concentration of agar on the wave frequency (N) of the different juvenile stages.

B - The effect of the concentration of agar on the velocity along axis (V_a) of the different juvenile stages.

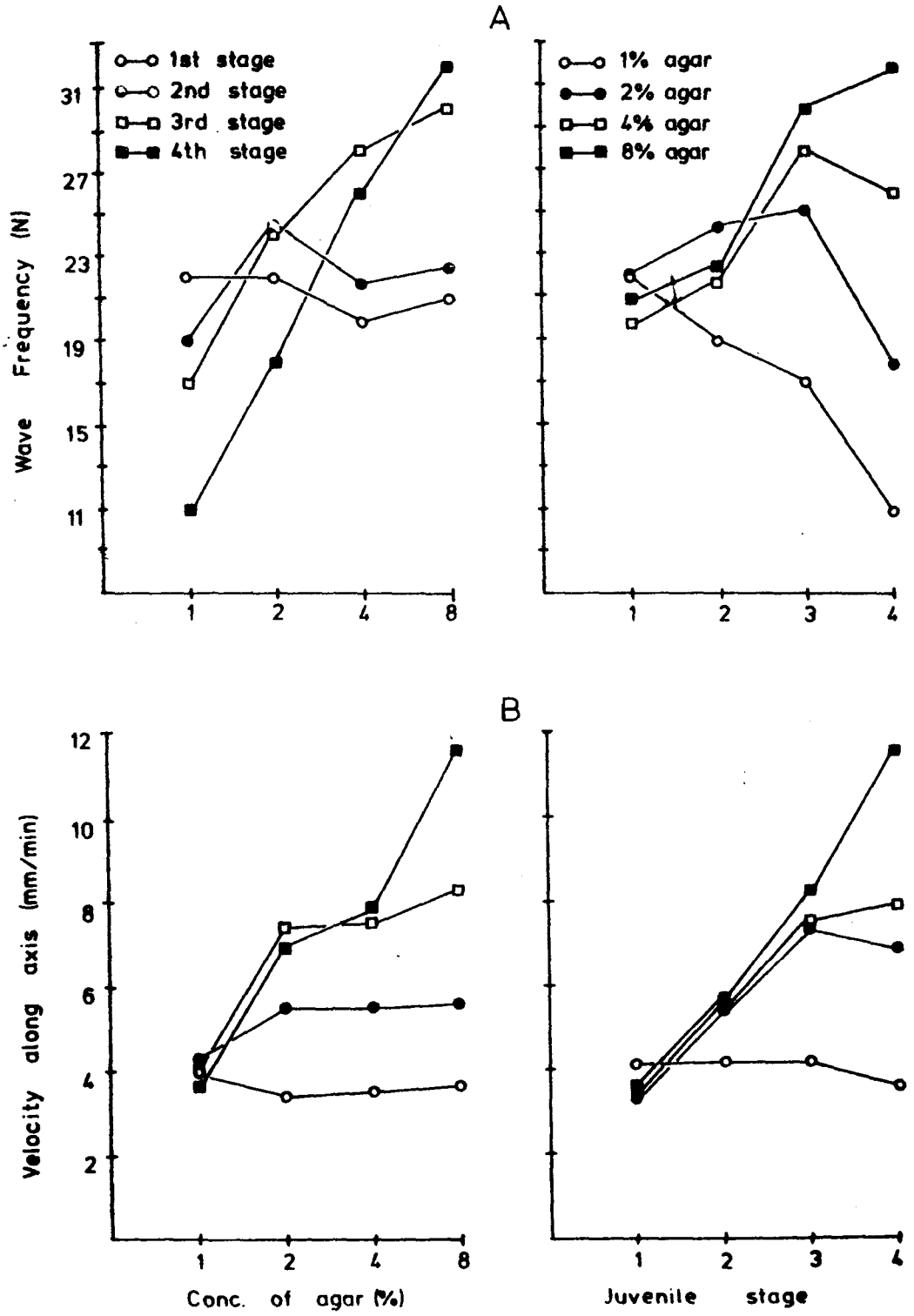

A
1st stage
2nd stage
3rd stage
4th stage
1% agar
2% agar
4% agar
8% agar
Wave Frequency (N)
31
27
23
19
15
11
1
2
4
8
1
2
3
4
B
Velocity along axis (mm/min)
12
10
8
6
4
2
1
2
4
8
Conc. of agar (%)
1
2
3
4
Juvenile stage

FIG. 49

was a slight decline but in the second and third-stage the speed increased as the concentration of the agar increased (Fig. 49,B). The speed of all stages in 1% agar was almost the same but it increased as the agar concentration increased (Fig. 49,B). The speed of all stages remained somewhat constant because V_a was dependant on wave frequency and the latter decreased as the juvenile stages advanced (Fig. 49,A). Similarly, an increase in the wave frequency coupled with a greater wave length of older juvenile stages produced greater velocity.

The locomotory behaviour of C. symmetricus juveniles was not similar in all stages but showed a gradual transition from the lower stage to the higher one. While the locomotory characteristics between the first and fourth-stage juveniles were usually well marked and often in sharp contrast to each other, those of the second and third-stages were somewhat similar and at times overlapped. In contrast to Meloidogyne javanica where all the parameters decreased with an increase in agar concentration, C. symmetricus juveniles showed a reversed trend. Such differences probably arose from differences in endogenous activity patterns being greater in the saprophagous species as well as in the nature of the two nematodes. Hence, in the absence of host roots, conservation of energy would be of utmost importance in the parasitic species while in the free-living species energy conservation would not be of survival value.

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DEVELOPMENTAL BIOLOGY OF *CHILOPLACUS SYMMETRICUS*

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The developmental biology of *Chiloplacus symmetricus* was studied by culturing these nematodes in malt-peptone agar in petri dishes. The eggs are laid mostly in the single-celled stage and the cleavage starts about an hour after egg laying. The cells in the anterior part are hyaline and divide more rapidly than those in the posterior part. Invagination begins about a day after laying and the juvenile hatches after about another 48 hours. During the life cycle there are four moults. The juvenile stages can be distinguished chiefly on the basis of their body size and the developing gonad. The two sexes can be distinguished from the second moult onwards. Cells forming the reproductive system divide only during the moulting period.

Free-living nematodes have been extensively used as an important tool in biochemical and nutritional studies (Dougherty *et al.*, 1959 ; Nicholas *et al.*, 1959 ; Vanfleteren, 1973). Their relatively short life cycles and high reproductive rates are an added advantage in these studies. However, developmental biology of only a few species has been studied. Chuang (1962) studied embryogenesis and post-embryogenesis in *Rhabditis teres* and Thomas (1965) gave an account of the life cycle of *Acrobeles complexus*. Chin and Taylor (1969, '70) and Chin (1977) have made an excellent study of the biology of *Cylindrocorpus* sp. Some aspects of the nutrition of the free-living nematode, *Chiloplacus lentus* were studied by Roy (1973a, '73b) who also described intra-uterine egg development in this species. Recently, Jairajpuri and Azmi (1977) gave a good account of the reproductive behaviour of *Acrobeloides* sp. The present study deals with the developmental biology of *Chiloplacus symmetricus* (Thorne, 1925) Thorne, 1939.

MATERIALS AND METHODS

Preparation of the culture media

The nematodes were cultured in malt-peptone agar in 5 cm dia. petri-dishes. The medium consisted of 2.5 gm of Difco malt, 2.5 gm of bacteriological peptone and 10 gm of Oxoid agar dissolved in 1000 ml of water. This medium

was then poured into petri-dishes and allowed to cool. The nematodes were innoculated into the agar with the help of a bamboo splinter or a suspension of nematodes was placed on the surface in a small drop of water. No attempt was made to sterilize the medium or the nematodes. This soon resulted in a luxuriant growth of bacteria on the surface of the agar upon which the nematodes fed actively. All specimens used in the present study were the progeny of a single gravid female.

Handling of nematodes

When required, the nematodes were recovered from the culture by a modified Baermann's funnel technique. The agar was cut into small pieces and placed on a sieve lined with moist tissue paper. The majority of the nematodes reached the bottom of the funnel within 24 hours. Picking was done with the help of a needle or bamboo splinter but when large numbers were required, the apparatus designed by Khan *et al.* (1972) was used.

Observation chambers

Embryonic development was studied in hanging drops of water. For the study of post-embryonic development a chamber similar to that designed by Maertens (1975) was used. A plastic ring 1 cm in dia. was fixed on a metallic slide and a cover-slip was fixed on one side. A 1 mm thick layer agar piece was cut in the form of a disc and placed on the coverslip. Males, females or juveniles were placed on the agar and another coverslip was placed over it and its edges were sealed with vaseline.

Staining

To study the development of the gonad of the nematode, live adults and the developing stages were directly immersed in 1% acetic orcein. The staining enables proper distinction of the various kinds of nuclei. The cytoplasm doesn't take up the stain. For observation at higher magnification the specimens were mounted in a drop of dilute stain.

Preparation of slides

Permanent mounts were prepared in anhydrous glycerine and temporary mounts in 4% formaldehyde solution or in water.

Observations and drawings

All observations were made under a stereoscopic binocular or a compound microscope. The drawings were made with the help of camera lucida. For taking measurements an ocular micrometer was used (See Table 1 for dimensions).

EMBRYOGENESIS

Egg

Freshly laid eggs measure 93 x 44 μm (79-108 x 40-50). They have a smooth shell, a dense granular cytoplasm which displays a characteristic streaming, and a fairly large nucleus. A polar body is usually visible between the cytoplasm and the vitelline membrane. Occassionally, a second polar body may also be visible. Usually, the egg is in the single cell stage when laid, but, in older females due to weakening of the uterine muscles, the eggs are retained inside the uterus for a longer duration and consequently, may divide twice or thrice before laying. However, eggs only up to four-cell stage were seen in the uterus. Only one egg was present in the uterus at a time.

Fertilization

As soon as the mature oocyte reaches the uterus from the oviduct, penetration by the sperm takes place. In most cases, however, the fusion of male and female pronuclei takes place outside the body of female. The two pronuclei which are at first situated at opposite poles, reach the centre by moving towards each other in an opposed path. They then orient themselves slightly obliquely to the longitudinal axis and fuse with each other. Subsequently, the cytoplasm retracts from the shell membrane and the vitelline membrane collapses as the fertilized egg prepares for cleavage.

Cleavage

Nearly an hour after fertilization, the first cleavage furrow appears prependicular to the long axis of the egg and divides it into two unequal blastomeres A and B, the former smaller than the latter. Cytoplasmic streamings cease at this point. After 2-3 hours, the second cleavage furrow appears parallel to the first one and divides the A blastomere into two equal cells A_1 and A_2. The resulting three cells are arranged at tandem, but usually a slight rotation causes A_1 and A_2 to orient obliquely. After a lapse of another 2-3 hours. A_1 divides along the longitudinal plane. The smaller of the two resultant cells again divides making a

TABLE I

Dimensions of adults and juveniles of Chiloplacus symmetricus (Mean values in parenthesis)

Stage numbers measured	L_1 10	L_2 10	L_3 ♂ ♂ 10	L_3 ♀ ♀ 10	L_4 ♂ ♂ 10	L_4 ♀ ♀ 10	Adult ♂ ♂ 15	Adult ♀ ♀ 15
Body length	224–317 µm	393–446 µm	468–562 µm	448–557 µm	599–727 µm	594–740 µm	813–994 µm	862–1102 µm
L	(282)	(421)	(523)	(499)	(653)	(667)	(864)	(926)
a	18–21	20–24	19–25	19–24	21–28	20–26	22–26	21–29
	(19)	(22)	(22)	(22)	(24)	(23)	(24)	(24)
b	2.3–2.5	2.6–3.0	2.8–3.2	2.8–3.2	3.0–3.5	3.0–3.5	3.4–4.0	3.4–4.3
	(2.4)	(2.8)	(3.0)	(3.0)	(3.2)	(3.3)	(3.8)	(3.7)
c	12–15	14–16	14–15	14–16	14–18	14–18	14–19	17–20
	(13)	(15)	(15)	(15)	(15)	(16)	(16)	(19)
V/T							42–52 (47)	65–71 (68)
Dist. from ant. end	64–73 µm	94–105 µm	101–109 µm	101–104 µm	109–116 µm	116–123 µm	129–159 µm	130–164 µm
to nerve ring	(68)	(101)	(103)	(102)	(112)	(120)	(138)	(151)
Gonad length	10–13 µm	14–15 µm	25–35 µm	22–31 µm	61–95 µm	68–105 µm	337–446 µm	234–400 µm
	(11)	(15)	(29)	(26)	(80)	(85)	(411)	(297)
Tail length	18–21 µm	27–31 µm	34–39 µm	29–38 µm	38–46 µm	38–47 µm	50–59 µm	46–55 µm
	(20)	(29)	(36)	(34)	(43)	(42)	(54)	(50)

total of five cells. 3-4 hours after the fourth cleavage, the B blastomere which has remained undivided so far, divides longitudinally into two almost equal cells B_1 and B_2. Of these two, B_2 moves upwards and comes to lie on the side of A_2. After 2-3 hours, A_2 divides transversely. Further observations on the cleavage become difficult because of rapid divisions, constant movement of the cells and also due to their dense granulation. The ensuing rapid divisions produce a mass of cells which represent the 'morula stage', almost a day after the first cleavage. A few hours later the cellular differentiation becomes more obvious resulting in the demarcation of the ectoderm and an endoderm. Synchronous with these developments, the cells in one half which is destined to become the anterior end appear hyaline, while those in the other half show a dense granular cytoplasm.

The formation of an invagination slightly away from the centre initiates the 'tadpole stage' of the embryo. 5-6 hours later, the embryo begins to show movements. At first, there is a slight movement confined to the cephalic region only but this gradually spreads over the entire body. This results in a spasmodic activity of the embryo within the egg shell. The first structure to make its appearance is the oesophago-intestinal junction. Concurrently, there is delineation of the intestinal wall from the ectoderm and the formation of the rudiments of the oesophageal bulb. Movements at this stage are sluggish and occasional.

Nearly eight hours after the tadpole stage, the embryo assumes a length which is more than $2\frac{1}{2}$ times of the length of the egg. While the differentiation of the intestine proceeds from posteriorly, the oesophagus becomes differentiated throughout its length. The rhabdions and the oesophageal lumen appear later. 15-18 hours after the initiation of invagination, the cephalic and labial probolae make their appearance, the cuticularization of the oesophageal lumen begins and the intestine is completely formed. The rectum begins to show at this stage as a broad hollow tube. One to two hours later, the stoma appears as two parallel and closely placed hyaline plates. At this point, the formation of the rectum is completed and the cuticularization of its lumen proceeds from anus to intestine. The complete formation of the oesophagus along with its lumen and valvular apparatus and the differentiation of the stomal rhabdions occurs 3-4 hours later. The intestine also develops at the same time. At this stage the juvenile begins to move actively back and forth, or revolves along its longitudinal axis. Activity within the egg is always interrupted for short periods of quiescence.

Hatching

Although it could not be ascertained, there is a possibility that the first stage juvenile moults once while already inside the egg. Exsheathment was not

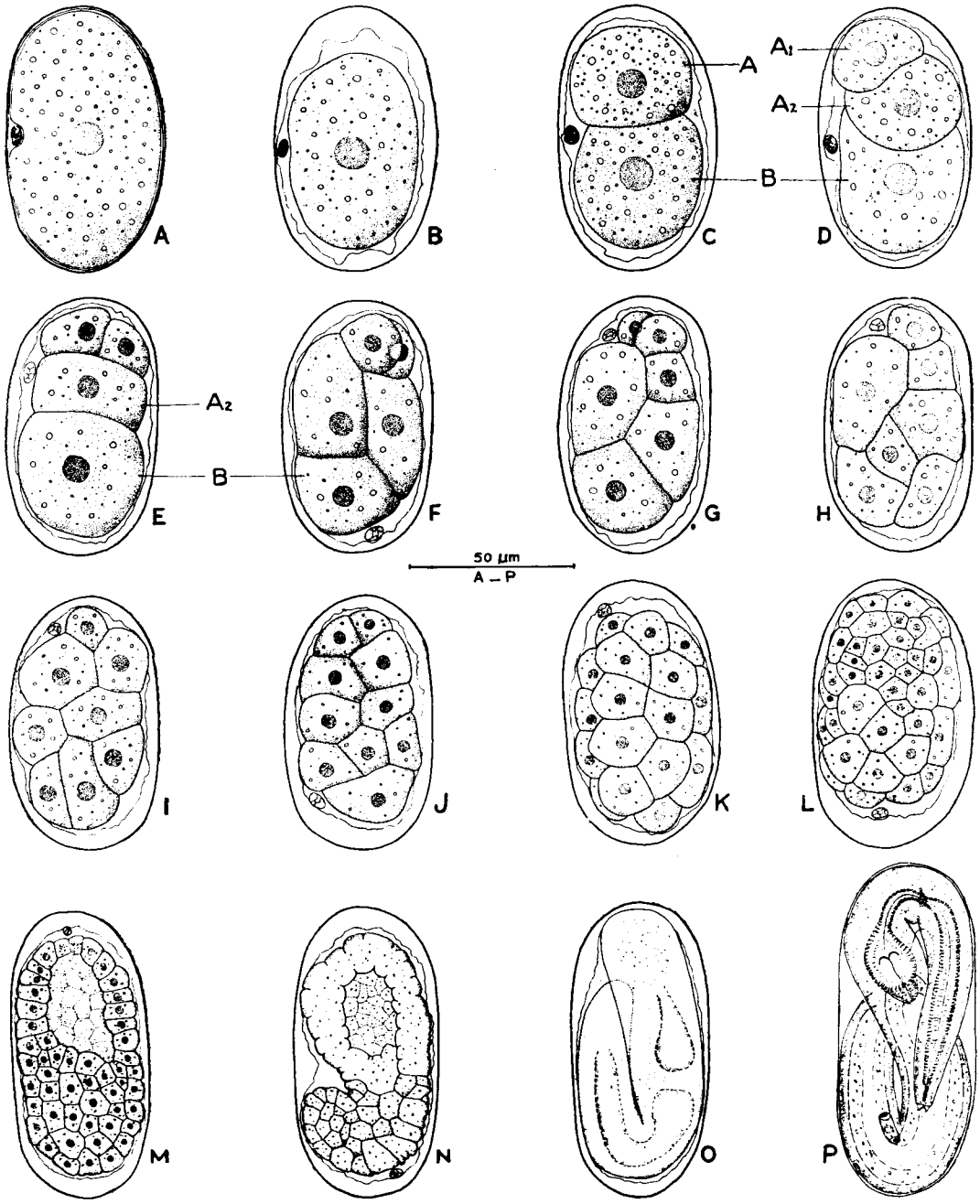

Fig. 1. Embryonic development : A—Fertilized egg showing one polar body, B—Retraction of cytoplasm prior to cleavage, C—Two cell stage, D—Three cell stage, E—Four cell stage, F—Five cell stage, G—Six cell stage, H—Seven cell stage, I—Eight cell stage. J—Ten cell stage, K—Early morula stage, L—Late morula stage, showing differentiation of cells, M—Gasrtulation, N—Invagination, O—Juvenile showing rudiments of oesophagus and intestine. P—Fully formed juvenile prior to hatching.

observed but the oesophageal lumen and the valvular apparatus were seen to pass into the intestine and were expelled through the anus, either just before or soon after hatching. Oesophageal pulsations start 6-7 hours before hatching. At first they are sporadic, occurring in short bursts or at irregular intervals, but later becoming more persistent and rhythmic although still in irregular phases. The rate of oesophageal pulsations was used for forecasting hatching in *Acrobeles complexus* (Thomas, 1965) and *Acrobeloides* sp. (Jairajpuri and Azmi, 1977). However, no such phenomenon was observed in *Chiloplacus symmetricus* and the pulsations showed a peak soon after commencement, then there was a sudden fall and afterwards it continued at an uneven rate till hatching. Hatching took place nearly 72 hours after the egg laying. Prior to this the juvenile becomes very active showing an almost incessant movement. The egg shell displays considerable elasticity which becomes evident because of a continuous change in its contour due to movement. Hatching results because the juvenile continuously probes the shall with its head and also due to internal pressure caused by its growth. Soon upon hatching, the juvenile shows a tendency to recoil back into the shell. This may be due to the sudden change in the environment experienced by this young animal. Feeding starts soon thereafter and particulate matter can be seen entering the intestine of the animal.

First stage juvenile

In the first stage juvenile a small oval germinal primordium is situated at approximately 64% of the body length from the anterior end. It consists of a centrally located germinal nucleus flanked at the anterior and posterior ends by two somatic nuclei. Between the base of oesophagus and the germinal primordium, the ventral chord nuclei number 15-18 and their arrangement is irregular. This is a diagnostic character of the first stage juvenile.

The onset of the first moulting becomes apparent when the periods of inactivity become fairly prolonged. The feeding gradually stops and the juvenile enters a quiescent phase which may be interrupted by occasional twitchings of the head. The oesophageal pulsations also cease. The new cuticle appears at the lip region and as it develops further, the cheilorhabdions become fairly visible but the meso-, meta-, and telorhabdions become progressively fainter. The activity continues to be very limited and only slight back and forth movements and occasional sudden rotations of the body may be seen. A few hours later, the cuticle of the lip region separates taking along with it the stomal rhabdions. The cheilorhabdions of the new stoma become prominent, the other parts are formed later. The oesophageal lumen becomes indescernible and the valvular apparatus

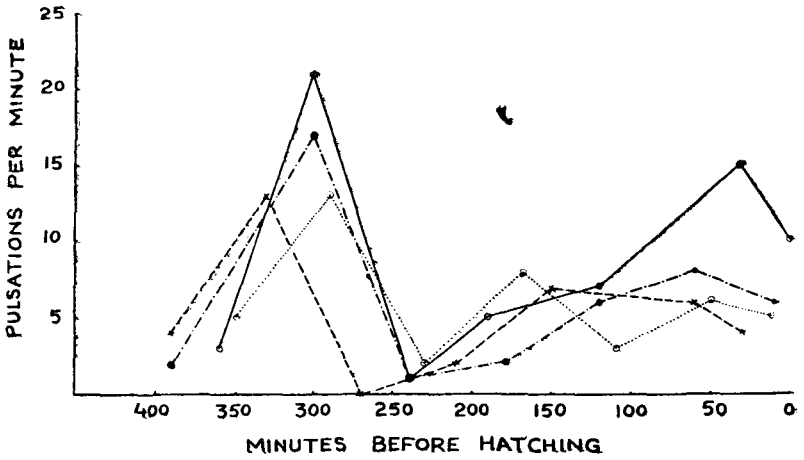

Fig. 2. Rate of oesophageal pulsations of four juveniles from start till hatching.

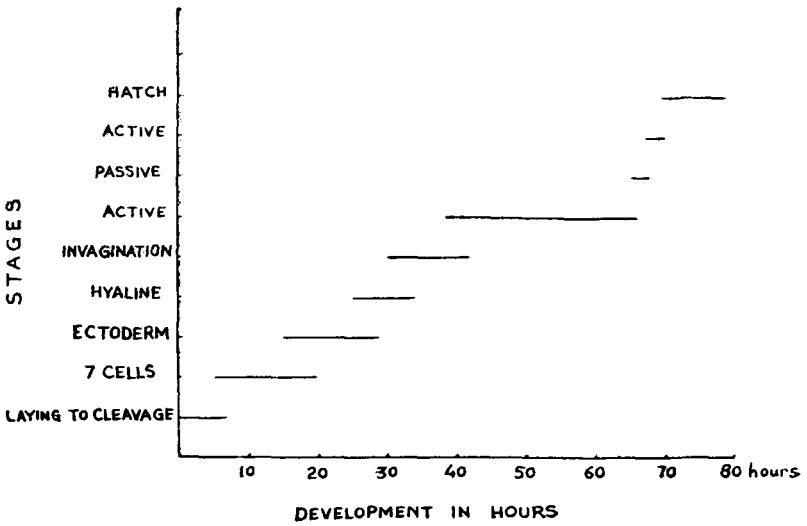

Fig. 3. Duration of various developmental stages.

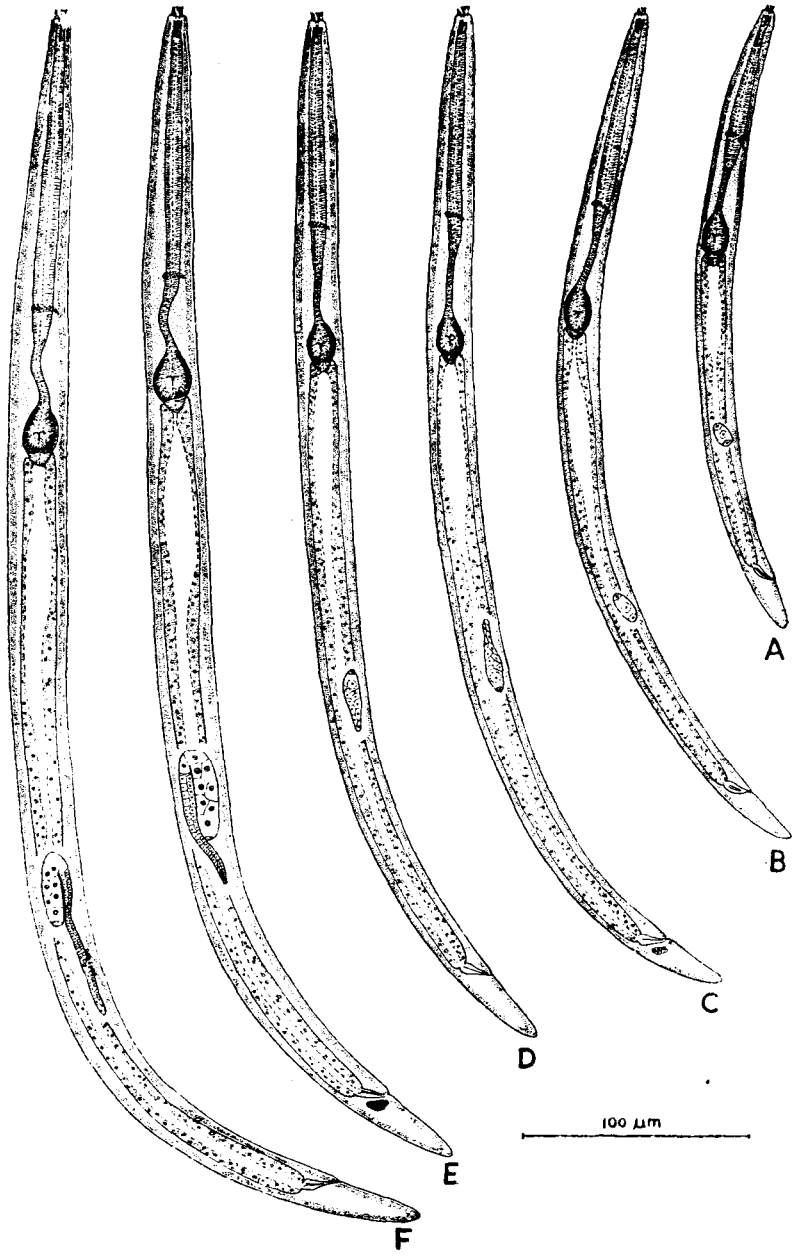

Fig. 4. Juvenile stages : A—Hatched first stage juvenile, B—Second stage juvenile, C—Third stage juvenile, male ; D—Third stage juvenile, female ; E—Fourth stage juvenile, male ; F—Fourth stage juvenile, female.

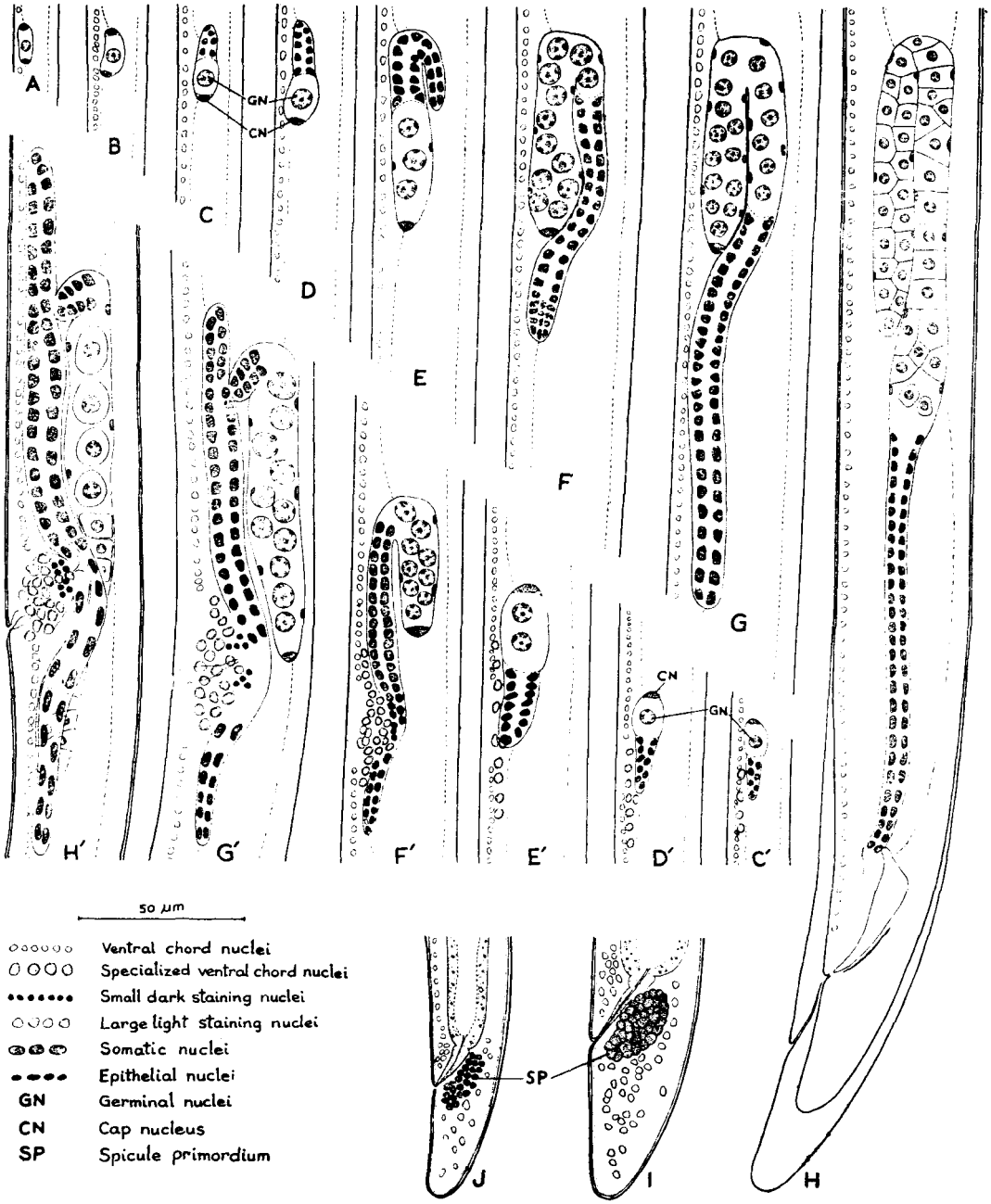

Fig. 5. Development of the gonad : A—First stage juvenile; B—Seeond stage juvenile ; C—Second moulting stage, male ; C'—Second moulting stage, female ; D—Third stage juvenile, male ; D'—Third stage juvenile, female ; E—Third moulting stage, male ; E'—Third moulting stage, female ; F—Fourth stage juvenile, male ; F'—Fourth stage juvenile, female ; G—Early fourth moulting stage, male ; G'—Early fourth moulting stage, female ; H—Late fourth moulting stage, male ; H'—Late fourth moulting stage, female ; I—Fourth stage male tail ; J—Third stage male tail.

becomes obscure. Concurrently, the cuticle in the tail also frees itself. The activity at this stage increases and the head swings sideways. The new stomal rhabdions become distinct and the oesophageal lumen gradually makes its appearance. With increasing activity the juvenile is able to completely free itself from the old cuticle. It appears that continuous probing of the lip region against the old cuticle, coupled with friction against the semi-solid agar surface (soil particles in nature) eventually causes the old cuticle to break at the anterior end and the juvenile escapes from the exuvium.

The only structural changes that occur during this moult are slight increase in the body length and multiplication and regular arrangement of the ventral chord nuclei.

Second stage juvenile

Very little morphological changes occur in the course of the first moult. The germinal primordium still consists of a large germinal cell and two smaller somatic cells as in the first stage. There are 34-36 ventral chord nuclei arranged in a single row between the base of oesophagus and the germinal primordium. In addition to body length, this being the only character separating the second stage juvenile from the first stage, there being no change in the germinal or somatic cells.

The first divisions within the germinal primordium occur at the time of second moulting. The cap nucleus divides repeatedly producing several nuclei which subsequently arrange themselves in patterns which are different in the sexes. As a result, the germinal nucleus is displaced anteriad if the juvenile is female, or posteriad if the juvenile is a male. Irrespective of the sex, only one somatic nucleus divides, the other remaining inactive throughout the developing period. In addition to the characteristic arrangement of the somatic nuclei in the primordium, the female juveniles also have four ventrally placed specialized ventral chord nuclei. Two of these nuclei are posterior to the primordium while the third one is near the posterior tip and the fourth one is at about the middle of the genital primordium. Except for the development of the gonad, the sequence of moulting is similar in all stages.

Third stage juvenile

Morphological changes between the sexes in the third stage become more pronounced. The male gonad consists of five pairs of somatic nuclei and a single large germinal nucleus besides a cap nucleus. The somatic nuclei which lie anterior to the germinal nucleus in the later stages, from the gonoduct of the

male reproductive system as well as the epithelium of the testis. Specialized ventral chord nuclei are typically lacking. In the anal region, dark staining and closely placed nuclei form the spicule primordium.

The female genital primordium shows a similar arrangement of cells, but in a reversed condition. Thus, the single germinal nucleus is situated anterior to the ten somatic nuclei. As in male, the somatic nuclei form the tubular structures of the female reproductive system as well as the epithelial layer of the ovary. The four specialized ventral chord nuclei of the early second moult multiply twice and result into eight nuclei which are arranged in such a way that two of these lie in close proximity and parallel to each other, at the posterior tip of the gonad while three are placed anterior and the rest three posterior to these. The linear arrangement of the ventral chord nuclei breaks at this stage. The break occurs near the posterior tip of the gonad just opposite the symmetrically placed specialized ventral chord nuclei and represents the future vaginal area.

The onset of the third moult initiates a period of intense cellular activity within the gonad. In male juveniles, the genital primordium grows up anteriorly, reflexes and then courses its way downwards. The somatic nuclei proliferate rapidly and the tip of the future gonoduct grows beyond the rudimentary testicular lobe. Division in the germinal nucleus occurs for the first time and the resulting nuclei continue to divide several times. Within the testicular lobe, the growing spermagonial cells are arranged in a single row but at the completion of the moult they assume a diagonal pattern. In the rectal area, the cells forming the spicule primordium multiply further and form a compact mass.

As in male juveniles, the female genital primordium also grows anteriorly with a rapid division of the somatic nuclei, but this growth is also accompanied by a posterior elongation. In juveniles, nearing the completion of the third moult, the anterior region along with the developing oogonial cells bends over posteriorly forming a small elongate-oval ovarian lobe. The eight specialized ventral chord nuclei of the second stage multiply further and start inward migration in preparation for their future role, *i.e.*, the formation of the vagina. Those anterior to the future vagina move inwards and then outwards laterally forming half the cylindrical area and the other half is formed by the posteriorly located specialized ventral chord nuclei. In addition to these, two other kinds of nuclei, both of different size and of different staining property, appear for the first time, wedged in between the specialized ventral chord nuclei.

Fourth stage juvenile

In the fourth stage juveniles there is considerable elongation in the length of the gonad. Male juveniles have a reflexed testis consisting of 15-22

spermagonial cells. The testicular zone is differentiated from the future gonoduct by the presence of a transverse membrane. The proceeding 30 somatic cells are arranged in pairs, beyond these there are 12 cells arranged in three rows.

Female juveniles have a characteristic dense accumulation of various types of nuclei in the vaginal area. At least three different kinds of nuclei are present. The specialized ventral chord nuclei aggregate round the vaginal region forming an oblique cylinder. However, complete migration is not yet accomplished and some of these nuclei are still arranged longitudinally along the mid-ventral axis ; their total number ranges from 24-26. The previously mentioned small, dark staining nuclei, now six in number are arranged at the distal end of the vagina forming a ring. The larger light staining nuclei are placed laterally at the proximal end, one pair on each side. The somatic cells which form the uterus, oviduct and post-uterine sac are arranged in pairs along the length. From the flexure of the ovarian lobe and proceeding downwards are 17 pairs of uterine nuclei which, in the region of vagina, bend dorsally. Twelve somatic nuclei form the post-uterine sac. In contrast to the 15-22 spermagonial cells present in the males, the females have only up to 12 developing oogonia.

Moulting fourth stage juveniles show a rapid growth of the gonad. Although posterior elongation is a result of rapid cell division, the anterior growth is caused due to distension and hypertrophy rather than hyperplasia. In males the somatic cells proliferate and arrange themselves in pairs along the length of the gonoduct while the testis is pushed upwards. The lumen of the vas deferens appears during the late stages of the moult, simultaneously with the detachment of the primary spermatocytes from the testis. By this time the distal end of the testis approximates the reflexed proximal tip. The spicules appear as faint refractory lines midway during the moult. Gradually they thicken, first at the head and distal end, and then in the middle. Simultaneously with the formation of the spicules, the gubernaculum appears as a faint structure but rapidly becomes cuticularized and is completed even before the spicules

In females, during the initial stages of the fourth moult, the junction of the uterus and oviduct grows anteriorly to form the spermatheca. At least 12 nuclei are present in the spermatheca and they cannot be distinguished in anyway from the somatic nuclei of the uterus. The ovary along with the developing oogonia grows downwards and at the completion of the moult, the tip of the ovary lies even beyond the distal end of post-uterine sac. The vaginal lumen becomes apparent at the onset of the fourth moult but the uterine lumen develops later. Specialized ventral chord nuclei which are arranged longitudinally, move towards the vagina and arrange themselves ventrally in a circular manner forming the

proximal part of the vagina. The distal part of the vagina has small dark staining nuclei forming a ring. They move apart slightly and become arranged in two groups of four. The larger nuclei formerly situated outwards laterally, move inwards and become arranged at the lateral side of the vagina perpendicular to the smaller nuclei. Towards the end of the moult, the vaginal lumen breaks into the uterine lumen. By this time the post-uterine sac becomes elongated attaining its natural size.

By the time the new cuticle is formed the preadult loosens itself from the old cuticle. The entire reproductive system of both the sexes is completely formed. Immediately upon emergence from the exuvium the nematode begins to feed actively.

DISCUSSION

The pattern of cleavage during the embryonic development of *Chiloplacus symmetricus* resembles that of *Acrobeles complexus* (Thomas, 1965) except for the fourth division. The polarity could be determined during the second division and it is the anterior blastomere which divides first. This is similar to *A. complexus* (Thomas, 1965) ; *Ditylenchus dipsaci* (Yuksel, 1960) ; *Seinura* sp. (Hechler and Taylor, 1966) ; *Pratylenchus* sp. (Roman and Hirschmann, 1969) and *Cylindrocorpus longistoma* (Chin, 1977). However, the subsequent divisions are different in each of the above species.

The shedding of the oesophageal lining while the juvenile is still within the egg shell indicates a possible moulting inside the egg shell but, after hatching, exsheathment occurs four times corresponding to the four developmental stages. Another peculiarity is that the genital primordium of the hatched first stage juvenile and the second stage juvenile is exactly similar. Chin (1977) also observed similarly in *C. longistoma* but he noticed no other developmental peculiarity It may be speculated that the shedding of the oesophageal lining of the first stage juvenile within the egg is in fact the initiation of the first moult which is completed outside the egg shell after hatching. The first moult in tylenchs also occurs within the egg but it is followed by only three moults outside the egg shell (Hirschmann, 1962 ; Fassuliotis, 1962 ; Hechler and Taylor, 1966 ; Jairajpuri, 1968 ; Roman and Hirschmann, 1969 and Siddiqui and Taylor, 1970). Moulting within the egg has not so far been reported in any other species of saprophagous spnematodes. Observations on the activity and oesophageal pulsations within the egg provide further evidence in favour of a moult within the egg. Graphic representation of the oesophageal pulsations shows a peak soon after the commencement and

then a sharp fall followed by irregular pulsations up to hatching. The time range for the minimum pulsations per minute, representing a period of inactivity, corresponds to the time when the cuticular lining of the oesophagus appears in the intestine. Although oesophageal pulsation rate increased as the hatching time approached in *A. complexus* (Thomas, 1965) and *Acrobeloides* sp. (Jairajpuri and Azmi, 1977), no such phenomenon was observed in *Chiloplacus symmetricus*. The pulsation rate followed a very unpredictable pattern with the approach of eclosion. Hatching occurred as a result of pressure on the egg shell due to growth of the embryo and also due to probings with the lip region. These probings were, however, not specifically localised and no blister formation occurred as in *C. longistoma* (Chin, 1977). Although the oesophageal bulb did beat regularly, no fluid was seen to pass through the oesophagus. However, enzymatic action cannot be ruled out because at the time of hatching, the shell became extremely thin and highly elastic.

The development of the gonad has been studied in a number of nematode species viz., *Ditylenchus triformis* (Hirschmann, 1962), *D. destructor* (Anderson and Darling, 1964), *Helicotylenchus dihystera* (Hirschmann and Triantaphyllou, 1967), *H. valgaris* (Yuen, 1965), *Pratylenchus* sp. (Roman and Hirschmann, 1969), *Hoplolaimus indicus* (Dasgupta *et al.*, 1970) and *C. longistoma* (Chin, 1977). In all these species there is a basic pattern of development. Hirschmann (1962) pointed out that the number of germinal nuclei present in the first stage may be one or two and is not predetermined by the number of gonads in the adult. The derivation of the cap nucleus in *Chiloplacus symmetricus* from the somatic nuclei during the second moult is similar to that of *D. triformis* (Hirschmann, 1962) and even the subsequent development is also strikingly similar. The division of the somatic nuclei and the subsequent displacement of the germinal nucleus in the third stage is characteristic of a monodelphic pattern of gonad development. The 'I' nucleus (Hirschmann and Triantaphyllou, 1967) is conspicuously lacking in *C. symmetricus* and the vaginal organization is carried out by the specialized ventral chord nuclei themselves. A break in the ventral chord nuclei occurs when the 'I' nucleus or the 'vaginal initial' (Anderson and Darling, 1964) moves in between them. In *C. symmetricus* this function is performed by the coming together of two medially placed specialized ventral chord nuclei. Chin, (1977) did not mention 'I' nucleus but has described a 'granular triangular somatic nucleus' which behaved in a manner similar to the 'vaginal initial'. The 'I' nucleus and the 'vaginal initial' are probably homologous.

The specialized ventral chord nuclei form the main components of the vagina. Their appearance early in the second moult helps to distinguish

the male and female, juveniles. Besides these, additional nuclei are also found in the vaginal area. The small dark staining nuclei appearing late in the third moult forming a ring around the distal end of the vagina in the fourth stage could correspond to the 'nuclei outside gonad' (*cf.* Hirschmann, and Triantaphyllou, 1967). The light staining nuclei placed laterally at the proximal tip of the vagina are also probably modified nuclei derived from the specialized ventral chord nuclei.

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SHORT COMMUNICATIONS

RECORD OF AN INTERSEX OF *AQUATIDES THORNEI* WITH REMARKS ON THE PHENOMENON OF INTERSEXUALITY IN NEMATODES

BY

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Soil sample collected from around the roots of paddy, *Oryza sativa* L., at Bodh Gaya near Magadh University Campus, Bihar, India, yielded one normal female, one normal male and an intersex of *Aquatides thornei*. This is the first record of the species from India and also the first report of an intersex in a nygolaimoid nematode belonging to the Order Dorylaimida.

AQUATIDES THORNEI (SCHNEIDER, W., 1937)

Dimensions

Normal female : L=1.62 mm ; a=32 ; b=4.1 ; c=60 ; V=49 ; G_1=9 ; G_2=12 ; Mural tooth=14 μm ; oesophagus=388 μm ; tail=27 μm.

Normal male : L=1.42 mm ; a=32 ; b=3.9 ; c=54 ; T=59 ; Mural tooth=13 μm ; oesophagus=360 μm ; tail=26 μm ; spicule=43 μm ; gubernaculum=8 μm ; lateral guiding pieces=10 μm ; ventromedian supplements=5 ; copulatory muscles=25.

Intersex : L=1.60 mm ; a=31 ; b=4.4 ; c=59 ; V=49 ; G_1=8 ; G_2=12 ; T=15 ; Mural tooth=13 μm ; oesophagus=360 μm ; tail=27 μm ; spicule=27 μm ; lateral guiding pieces=7 μm ; ventromedian supplements=6 ; copulatory muscles=28.

Descriptions

Normal female : Body almost straight upon fixation. Lip region continuous with body contour. Mural tooth about one head-width long. Basal expanded part of oesophagus occupying about 59% of oesophageal length. Five conspicuous oesophageal gland nuclei present. Cardiac

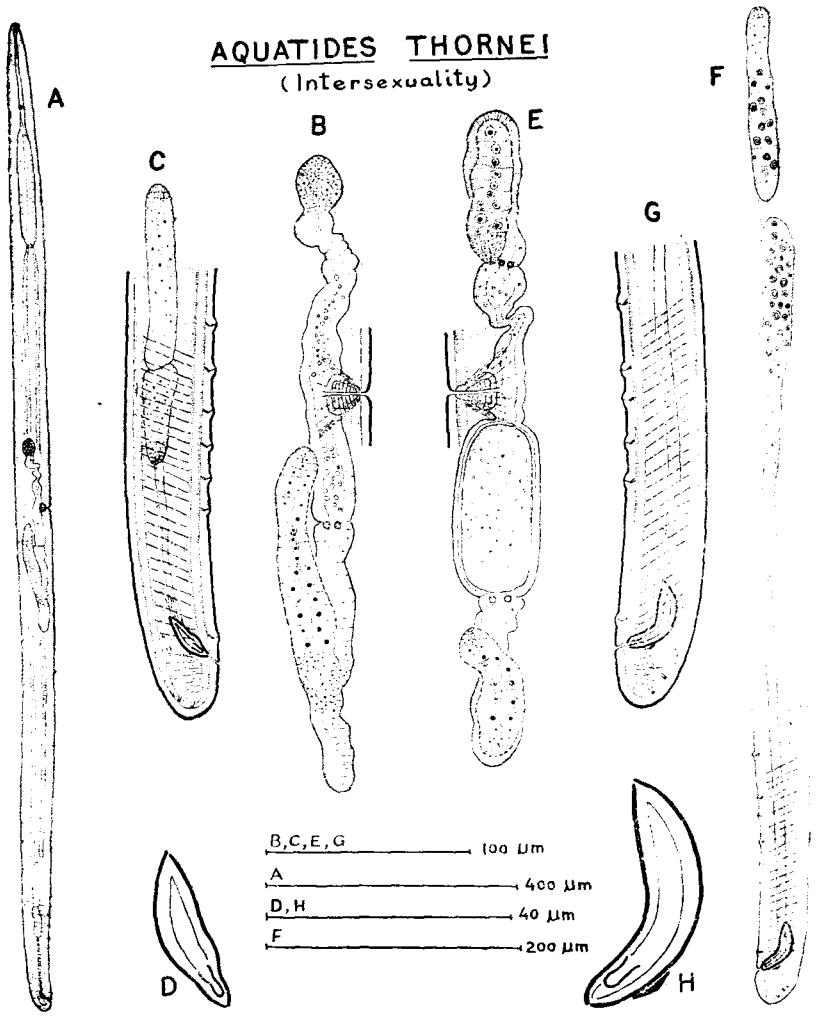

Fig. 1 : A-H. *Aquatides thornei* : A—Intersex, entire; B—Female reproductive system of intersex ; C—Posterior region of intersex showing male reproductive system ; D—Copulatory apparatus of intersex ; E—Reproductive system of normal female ; F—Reproductive system of normal male; G—Posterior region of normal male ; H—Copulatory apparatus of normal male.

glands rounded, 7 x 9 μm in size. Reproductive system amphidelphic. Vulva transverse, supported by vulval muscles. Ovaries reflexed, oocytes arranged in multiple rows. Prerectum about 60 μm or about two anal body-widths long, and rectum slightly less than one anal body-width long. Tail hemispheroid, slightly clavate.

Normal male : Body shape as in female. Reproductive system diorchic, one testis reflexed, other outstretched. Supplements consist of an adanal pair and five ventromedians. Spicules massive, ventrally curved, 43 μm or about 1.6 anal body-widths long medially. Lateral guiding pieces tongue-shaped, 10 μm long. Gubernaculum slender, 8 μm long. Copulatory muscles 25, extending a little short of last ventromedian supplement. Tail hemispherical.

Intersex : Body almost straight upon fixation as in normal sexes. Lip region continuous with body contour. Mural tooth about one head-width long. Basal expanded part of oesophagus occupying about 53% of oesophageal length. Five conspicuous oesophageal gland nuclei present. Cardiac glands rounded, 8 x 9 μm in size.

Female reproductive system amphidelphic. Vulva transverse, supported by vulval muscles. Anterior ovary outstretched, degenerate with only a few germinal cells ; posterior ovary reflexed, normally developed with its oocytes arranged in multiple rows.

Male reproductive system with genital ducts poorly developed. Testes rudimentary, one reflexed and the other outstretched. Poorly developed germinal cells present at the tip of each testis. Spicules straight, 27 μm or about one anal body-width long medially. Supplements consist of an adanal pair and six well developed ventromedians. Lateral guiding pieces small, 7 μm long. Gubernaculum absent. Copulatory muscles 28, extending a little short of the last ventromedian supplement. Tail hemispherical.

Remarks : The intersex possesses dominating female characters. It has normal femal gonads except that the anterior gonad is a little degenerate with few germinal cells but it may still be functional. The anterior ovary is short and outstretched while the posterior ovary is fully developed. The male reproductive system is degenerate and perhaps non-functional. Testes are very small and without spermatocytes. Only a few poorly developed germinal cells are present at the tip of each testis. The spicules are smaller than in the normal male and also of a different shape with pronounced narrowing towards the tip. Lateral guiding pieces are small and the gubernaculum is absent. However, the ventro-median supplements and the copulatory muscles are as well developed as in the normal male. In fact, the number of ventromedian supplements and the copulatory muscles are a little more in number in the intersex than in the normal male.

INTERSEXUALITY IN NEMATODES

Intersexuality in nematodes is not of rare occurrence as was thought by Jairajpuri and Siddiqi (1964). It has been reported in many nematode species belonging to different habitats and comprising of diverse groups (Table I). According to Mayr (1969) intersexes may arise from an imbalance between male tendency and female tendency genes resulting from irregularities in fertilization or mitosis or physiological disturbances associated with parasitism and as such they are more likely to occur in sympatric species or subspecies as a result of hybridization. While such a hybridization of closely related genotypes may cause intersexuality in mermithids (Steiner, 1923) and *Aphelenchoides* (Krall, 1971), incomplete sex reversal under unfavourable conditions may produce intersexes in species of *Meloidogyne* and other nematodes (Triantaphyllou, 1960 ; Hyman, 1951). Triantaphyllou (1971) believed that in polyploid and parthenogenetic species of *Meloidogyne* and *Meloidodera* the environment is more likely to effect sex expression. Further, polyploidy and

TABLE I

Reports of intersexuality in different nematode groups

Species	Habitat	Order	Author (s)	Year
1. *Hexamermis albicans*	Soil-inhabiting/ entomophagous	Mermithida	Meissner	1853
2. *Enoplus communis*	Marine	Enoplida	Schneider, A.	1866
3. *Porrocaecum heteroura*	Vertebrate host	Ascarida	Willemoes-Suhn	1869
4. *Chromadora poecilisoma*	Marine	Chromadorida	De Man	1893
5. *Thoracostoma figuratum*	Marine	Enoplida	De Man	1893
6. *Tobrilus* (=Trilobus) *gracilis*	Free-living	Enoplida	Linstow	1903
7. *Tobrilus* (=*Trilobus*) *diversipapillatus*	Free-living	Enoplida	Daday	1905
8. *Tobrilus* (=*Trilolus*) *gracilis*	Free-living	Enoplida	Ditlevesen	1911
9. sp. of mermithid	Soil-inhabiting/ entomophagous	Mermithida	Hagmeier	1912
10. *Tobrilus* (=*Trilobus*) *gracilis*	Free-living	Enoplida	Schneider, W.	1922
11. sp. of mermithid	Soil-inhabiting/ entomophagous	Mermithida	Steiner	1923
12. sp. of mermithid	Soil-inhabiting/ entomophagous	Mermithida	Christie	1929
13. *Meloidogyne javanica*	Plant-parasitic	Tylenchida	Chitwood, B. G.	1949
14. *Ditylenchus triformis*	Plant-parasitic	Tylenchida	Hirschmann & Sasser	1955
15. *Aphelenchoides parietinus*	Plant-parasitic	Tylenchida	Krall, E.	1959
16. *Meloidogyne javanica*	Plant-parasitic	Tylenchida	Triantaphyllou	1960
17. *Tyleptus striatus*	Soil-inhabiting	Dorylaimida	Jairajpuri & Siddiqi	1964
18. *Longidorus macrosoma*	Plant-parasitic	Dorylaimida	Aboul-Eid & Coomans	1966
19. *Telotylenchus* sp.	Plant-parasitic	Tylenchida	Dalmasso	1966
20. *Tylenchorhynchus nilgiriensis*	Plant-parasitic	Tylenchida	Seshadri *et al.*	1967
21. *Longidorus africanus*	Plant-parasitic	Dorylaimida	Cohn & Mordechai	1968
22. *Meloidogyne incognita*	Plant-parasitic	Tylenchida	Varma *et al.*	1971
23. *Aphelenchoides* sp.	Plant-parasitic	Tylenchida	Khera & Chaturvedi	1971
24. *Leptonchus obtusus*	Soil-inhabiting	Dorylaimida	Goseco & Ferris	1973
25. *Xiphinema ingens*	Plant-parasitic	Dorylaimida	Lamberti *et al.*	1975
26. *Xiphinema insigne*	Plant-parasitic	Dorylaimida	Bajaj & Jairajpuri	1977
27. *Aquatides thornei*	Soil-inhabiting	Dorylaimida	Jairajpuri *et al.*	1979

All are female intersexes except numbers 13, 16, 22 & 26 which are male intersexes.

aneuploidy may weaken the genetic mechanism of sex determination in these nematodes thus equalizing male and female factors in their chromosomal compliment. Chitwood (1950) related intersexuality to crowding and contended that a single female in the presence of numerous males may be induced to become an intersex.

As is evident from the above discussion, divergent views have been expressed about the factors responsible for causing intersexuality in nematodes. We feel that with a few exceptions, neither the environment nor hybridization between closely related species or subspices is the real cause of intersexuality in the majority of cases that occur in nature. The possibility that the (external) environment is the causitive factor is not tenable because an environmentally mediated phenomenon should effect the entire population of a particular species at a particular time rather than just an odd individual as is usually the case in most of the reports on intersexuality. Hybridization can also be ruled out because it involves the presence of at least two or more closely related species or subspecies in a particular habitat and this has not been reported by the authors who have recorded intersexes (Table I). In our opinion, in all probability, it is something within the animal itself (internal environment) which acts at the time of embryogensis to make it an intersex. As such the possibility of some genetic disorder upsetting the total maleness or femaleness of an individual belonging to a natural population of a nematode species is most likely to be the main cause of this phenomenon.

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